updated search 7/06

WEST Search History

Hide Items | Restore | Clear | Cancel

DATE: Monday, July 31, 2006

Hide?	Set Name	Query	Hit Count
		DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI,TDBD; PLUR=YES; OP=OR	
☐	L1	catalase.clm. and (pylori or pyloris or pylordis or pylon or pylorum or helicobacter).ti, ab,clm.	23

END OF SEARCH HISTORY

WEST Search History

Hide Items | Restore | Clear | Cancel

DATE: Monday, July 31, 2006

Hide?	Set Name	Query	Hit Count
		DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI,TDBD; PLUR=YES; OP=OR	
☐	L1	g-1-4 or g1-4 or g14	2771
☐	L2	L1 and (helicobacter or pylori or pyloris or pyloridis or hpylori or h-pylori)	101
☐	L3	L1 same (helicobacter or pylori or pyloris or pyloridis or hpylori or h-pylori)	12

END OF SEARCH HISTORY

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Search Results - Record(s) 1 through 12 of 12 returned.

1. 20050255043. 08 Apr 05. 17 Nov 05. Bacteriophage imaging of inflammation. Hnatowich, Donald J., et al. 424/9.1; 435/5 C12Q001/70 A61K049/00.

2. 20040138415. 03 Mar 04. 15 Jul 04. Helicobacter proteins, nucleic acids and uses thereof. Tian, Jing-Hui, et al. 530/350; C07K001/00 C07K014/00 C07K017/00.

3. 20020107368. 06 Dec 00. 08 Aug 02. Helicobacter proteins, gene sequences and uses thereof. Tian, Jing-Hui, et al. 530/388.4; 424/190.1 530/350 536/23.7 A61K031/70 C07H021/04 C07K001/00 C07K014/00 C07K017/00 C07K016/00 C12P021/08.

4. 6083683. 12 Jan 99; 04 Jul 00. Methods for detecting shigella bacteria or antibodies to shigella bacteria with an immunoassay. Pace; John Lee, et al. 435/4; 424/282.1 424/93.4 435/252.1 435/29 435/34 435/822 435/975. C12Q001/00 .

5. 6077678. 27 Jan 99; 20 Jun 00. Methods for detecting Campylobacter bacteria or antibodies to Campylobacter bacteria with an immunoassay. Pace; John Lee, et al. 435/7.1; 424/282.1 424/802 424/93.1 424/93.4 435/243 435/252.1 435/7.2 435/822 435/960 435/975. A61K045/00 C12N001/00 C12N001/12 G01N033/53 .

6. 6051416. 29 May 97; 18 Apr 00. Methods for producing enhanced antigenic Helicobacter sp.. Pace; John Lee, et al. 435/252.1; 424/184.1 424/234.1 424/93.1 435/822 435/960 435/961 435/975. A01N063/00 A61K039/00 C12N001/00 C12N001/12 .

7. 5976525. 07 Apr 97; 02 Nov 99. Method for producing enhanced antigenic enteric bacteria. Pace; John Lee, et al. 424/93.4; 424/282.1 435/252.1 435/29 435/30 435/34 435/38 435/7.1 435/822. A01N063/00 A61K045/00 C12N001/12 C12Q001/04 .

8. 5897475. 03 Oct 95; 27 Apr 99. Vaccines comprising enhanced antigenic helicobacter spp.. Pace; John Lee, et al. 435/252.1; 424/184.1 424/282.1 424/93.4. A01N063/00 A61K039/38 A61K045/00 C12N001/20 .

9. 5869066. 30 May 97; 09 Feb 99. Vaccine containing a campylobacter bacterium having an enhanced antigenic property. Pace; John Lee, et al. 424/282.1; 424/802 424/93.1 424/93.4 435/252.1 435/822. A01N063/00 C12N001/20 .

10. 5858352. 30 May 97; 12 Jan 99. Vaccine containing a Shigella bacterium having an enhanced antigenic property. Pace; John Lee, et al. 424/93.4; 424/184.1 424/252.1 424/282.1 435/822. A01N063/00 A61K045/00 C12N001/00 C12N001/20 .

11. 5681736. 03 Oct 95; 28 Oct 97. Methods for producing enhanced antigenic shigella bacteria and vaccines comprising same. Pace; John Lee, et al. 435/252.1; 424/184.1 424/282.1 424/93.4. A01N063/00 A61K039/00 A61K045/00 C12N001/20 .

12. 5679564. 03 Oct 95; 21 Oct 97. Methods for producing enhanced antigenic campylobacter bacteria and vaccines. Pace; John Lee, et al. 435/252.1; 424/184.1 424/282.1 424/93.4. A01N063/00 A61K039/38 A61K045/00 C12N001/20 .

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Term	Documents
HELICOBACTER	8548
HELICOBACTERS	40
PYLORI	8621
PYLORIS	224
PYLORIS	224
PYLORI	8621
PYLORIDIS	119
PYLORIDI	0
HPYLORI	5
HPYLORIS	0
H-PYLORI	27
(L1 SAME (HELICOBACTER OR PYLORI OR PYLORIS OR PYLORIDIS OR HPYLORI OR H-PYLORI)).PGPB,USPT,USOC,EPAB,JPAB,DWPI,TDBD.	12

There are more results than shown above. Click here to view the entire set.

Prev Page Next Page Go to Doc#

Search	Most Recent Queries	Time	Resu
#4	Search **g1-4 pylori**	13:50:58	
#6	Search **g-1-4 pylori**	13:50:46	
#5	Search **g14 pylori**	13:50:39	
#3	Search **g1-4 helicobacter**	13:50:29	
#2	Search **g14 helicobacter**	13:50:23	
#1	Search **bolin journal clinical microbiology**	13:43:41	

UniProtKB/Swiss-Prot entry Q9ZJ24

[Entry info] [Name and origin] [References] [Comments] [Cross-references] [Keywords] [Features] [Sequence] [Tools]

Note: most headings are clickable, even if they don't appear as links. They link to the user manual or other documents.

Entry information

Entry name	**YF88_HELPJ**
Primary accession number	**Q9ZJ24**
Secondary accession numbers	None
Integrated into Swiss-Prot on	October 18, 2001
Sequence was last modified on	May 1, 1999 (Sequence version 1)
Annotations were last modified on	May 2, 2006 (Entry version 23)

Name and origin of the protein

Protein name	**Hypothetical UPF0174 protein jhp_1494**
Synonyms	None
Gene name	OrderedLocusNames: jhp_1494
From	Helicobacter pylori J99 (Campylobacter pylori J99) [TaxID: 85963] [HAMAP proteome
Taxonomy	Bacteria; Proteobacteria; Epsilonproteobacteria; Campylobacterales; Helicobacteraceae; Helicobacter.

References

[1] NUCLEOTIDE SEQUENCE [LARGE SCALE GENOMIC DNA].
DOI=10.1038/16495; PubMed=9923682 [NCBI, ExPASy, EBI, Israel, Japan]
Alm R.A., Ling L.-S.L., Moir D.T., King B.L., Brown E.D., Doig P.C., Smith D.R., Noonan B
Guild B.C., deJonge B.L., Carmel G., Tummino P.J., Caruso A., Uria-Nickelsen M., Mills D
Ives C., Gibson R., Merberg D., Mills S.D., ↔, Trust T.J.;
"Genomic sequence comparison of two unrelated isolates of the human gastric pathogen Helicobacter pylori.";
Nature 397:176-180(1999).

Comments

- ***SIMILARITY***: Belongs to the UPF0174 family [view classification].

Copyright

Cross-references

Sequence databases

EMBL	AE001439; AAD07073.1; -; Genomic_DNA.	[EMBL / GenBank / DDBJ] [CoDingSequence]
PIR	B71800; B71800.	

3D structure databases

ModBase	Q9ZJ24.

Protein-protein interaction databases

DIP	Q9ZJ24.

Enzyme and pathway databases

BioCyc	HPYL85963:JHP1494-MONOMER; -.

2D gel databases

SWISS-2DPAGE	Get region on 2D PAGE.

Organism-specific gene databases

HOGENOM	[Family / Alignment / Tree]

Family and domain databases

InterPro	IPR005367; UPF0174. Graphical view of domain structure.
Pfam	PF03667; UPF0174; 1. Pfam graphical view of domain structure.
ProDom	[Domain structure / List of seq. sharing at least 1 domain]
BLOCKS	Q9ZJ24.

Genome annotation databases

GenomeReviews	AE001439_GR; jhp_1494.

Other

LinkHub	Q9ZJ24; -.

Genome annotation databases

CMR	Q9ZJ24; jhp_1494.

Other

ProtoNet	Q9ZJ24.
UniRef	View cluster of proteins with at least 50% / 90% / 100% identity.

Keywords

Complete proteome; **Hypothetical protein**.

Features

 Feature table viewer

Key	From	To	Length	Description	FTId
CHAIN	1	253	253	Hypothetical UPF0174 protein jhp_1494.	PRO_0000216424

Sequence information

Length: **253 AA** [This is the Molecular weight: **28476 Da** CRC64: **127158B2B1A2036A**

length of the unprocessed precursor] [This is the MW of the unprocessed precursor] is a checksum on the sequenc

```
        10         20         30         40         50         60
MAYKYDRDLE FLKQLESSDL LDLFEVLVFG KDGEKRHNEK LTSSIEYKRH GDDYAKYAER

        70         80         90        100        110        120
IAEELQYYGS NSFASFIKGE GVLYKEILCD VCDKLKVNYN KKTETTLIEQ NMLSKILERS

       130        140        150        160        170        180
LEEMDDEEVK EMCDELSIKN TDNLNRQALS AATLTLFKMG GFKSYQLAVI VANAVAKTIL

       190        200        210        220        230        240
GRGLSLAGNQ VLTRTLSFLT GPVGWIITGV WTAIDIAGPA YRVTIPACIV VATLRLKTQQ

       250
ANEDKKSLQI ESV
```

Q
in
F
fo

View entry in original UniProtKB/Swiss-Prot format
View entry in raw text format (no links)
Report form for errors/updates in this UniProtKB/Swiss-Prot entry

BLAST submission on ExPASy/SIB or at NCBI (USA)

Sequence analysis tools: ProtParam, ProtScale, Compute pI/Mw, PeptideMass, PeptideCutter, Dotlet (Java)

ScanProsite, MotifScan

Submit a homology modeling request to SWISS-MODEL

NPSA Sequence analysis tools

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CLUSTAL FORMAT for T-COFFEE Version_1.37, CPU=0.00 sec, SCORE=12700, Nseq=2, Len=253

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sp|Q9ZJ24|YF88_HELPJ           MAYKYDRDLEFLKQLESSDLLDLFEVLVFGKDGEKRHNEKLTSSIEYKRHGDD
                               **************************************************

unk|VIRT2629|Blast_submission  IAEELQYYGSNSFASFIKGEGVLYKEILCDVCDKLKVNYNKKTETTLIEQNML
sp|Q9ZJ24|YF88_HELPJ           IAEELQYYGSNSFASFIKGEGVLYKEILCDVCDKLKVNYNKKTETTLIEQNML
                               **************************************************

unk|VIRT2629|Blast_submission  LEEMDDEEVKEMCDELSIKNTDNLNRQALSAATLTLFKMGGFKSYQLAVIVAN
sp|Q9ZJ24|YF88_HELPJ           LEEMDDEEVKEMCDELSIKNTDNLNRQALSAATLTLFKMGGFKSYQLAVIVAN
                               **************************************************

unk|VIRT2629|Blast_submission  GRGLSLAGNQVLTRTLSFLTGPVGWIITGVWTAIDIAGPAYRVTIPACIVVAT
sp|Q9ZJ24|YF88_HELPJ           GRGLSLAGNQVLTRTLSFLTGPVGWIITGVWTAIDIAGPAYRVTIPACIVVAT
                               **************************************************

unk|VIRT2629|Blast_submission  ANGDKKSLQIESI
sp|Q9ZJ24|YF88_HELPJ           ANEDKKSLQIESV
                               ** *********:
```

Q9ZJ24
YF88_HELPJ

Hypothetical UPF0174 protein jhp_1494 [jhp_1494] [Helicobacter pylori J99 (Campylobacter pylori J99)]

Score = 493 bits (1270), Expect = e-138

Identities = 251/253 (99%), Positives = 252/253 (99%)

```
Query: 1   MAYKYDRDLEFLKQLESSDLLDLFEVLVFGKDGEKRHNEKLTSSIEYKRH
            MAYKYDRDLEFLKQLESSDLLDLFEVLVFGKDGEKRHNEKLTSSIEYKRH
Sbjct: 1   MAYKYDRDLEFLKQLESSDLLDLFEVLVFGKDGEKRHNEKLTSSIEYKRH

Query: 61  IAEELQYYGSNSFASFIKGEGVLYKEILCDVCDKLKVNYNKKTETTLIEQN
            IAEELQYYGSNSFASFIKGEGVLYKEILCDVCDKLKVNYNKKTETTLIEQN
Sbjct: 61  IAEELQYYGSNSFASFIKGEGVLYKEILCDVCDKLKVNYNKKTETTLIEQN

Query: 121 LEEMDDEEVKEMCDELSIKNTDNLNRQALSAATLTLFKMGGFKSYQLA
            LEEMDDEEVKEMCDELSIKNTDNLNRQALSAATLTLFKMGGFKSYQLA
Sbjct: 121 LEEMDDEEVKEMCDELSIKNTDNLNRQALSAATLTLFKMGGFKSYQLA

Query: 181 GRGLSLAGNQVLTRTLSFLTGPVGWIITGVWTAIDIAGPAYRVTIPACIV
            GRGLSLAGNQVLTRTLSFLTGPVGWIITGVWTAIDIAGPAYRVTIPACIV
Sbjct: 181  GRGLSLAGNQVLTRTLSFLTGPVGWIITGVWTAIDIAGPAYRVTIPACIV

Query: 241 ANGDKKSLQIESI 253
            AN DKKSLQIES+
Sbjct: 241 ANEDKKSLQIESV 253
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WEST Search History

Hide Items | Restore | Clear | Cancel

DATE: Monday, July 31, 2006

Hide?	Set Name	Query	Hit Count
		DB=PGPB,USPT,USOC,EPAB,JPAB,DWPI,TDBD; PLUR=YES; OP=OR	
☐	L1	tian.in. or walker.in. or jackson.in.	56560
☐	L2	L1 and (pylori or pyloris or pyloridis or pylorum or helicobacter or felis or hpylori or h-pylori)	106
☐	L3	l2 and (hp30 or hp-30 or (30 near (kda or daltons or rmw or mw)))	5
☐	L4	(hp30 or hp-30 or (30 near (kda or daltons or rmw or mw))).ti,ab,clm.	567
☐	L5	L4 and (pylori or pyloris or pyloridis or pylorum or helicobacter or felis or hpylori or h-pylori).ti,ab,clm.	15
☐	L6	lissolo.in. and l4	2

END OF SEARCH HISTORY

Publication Language: English
Filing Language: English
Fulltext Word Count: 12871

Fulltext Availability:
  Detailed Description

Detailed Description
... GenBank: M59426) and FLDB
  (GenBank: z48060) from Eschericia coli; FLDA (GenBank.

  AE001536 and AE000622) from %Helicobactar% pylori, and FLDA
  (GenBank: AE008840) from Salmonl1a typhlmurium which may
  improve the solubility and/or...nLinense
  IlCvlindrocarvon
  CYP55A3 nkinense @INOR2 CYLTO @@D78512
  -ho
  Ilsaccharomy-ces 97A.

  CYP56 i ICP56 YEAST @%55713% U32,
  cerevisiae
  ICYP57AI Ela:n:@i@EPID9 FUSSO 757
  ICYP57A2]IFusarium solani JOID6 FUSSO IX73145...

1/3,KWIC/2 (Item 1 from file: 348)
DIALOG(R)File 348:EUROPEAN PATENTS

00772536
METHODS FOR PRODUCING ENHANCED ANTIGENIC %HELICOBACTER% SP. AND VACCINES
  COMPRISING SAME
VERFAHREN ZUR PRODUKTION VON VERSTARKT ANTIGEN WIRKENDEN HELIOBACTER SP.
  UND VAKZINE DIE DIESEN ENTHALTEN
METHODES DE PRODUCTION D'%HELICOBACTER% SP. ANTIGENE AMELIORE ET DE VACCINS
  LE CONTENANT
PATENT ASSIGNEE:
  Antex Biologics, Inc., (1525991), 300 Professional Drive, Gaithersburg,
  MD 20879, (US), (Proprietor designated states: all)
INVENTOR:
  PACE, John L., 13117 Thackery Place, Germantown, MD 20874, (US)
  WALKER, Richard I., 120 Briscoe Street, Gaithersburg, MD 20878, (US)
  FREY, Steven M., 12529 Cross Ridge Way, Germantown, MD 20874, (US)
LEGAL REPRESENTATIVE:
  O'Connell, Maura (72391), F. R. Kelly & Co., 27 Clyde Road, Ballsbridge
  , Dublin 4, (IE)
PATENT (CC, No, Kind, Date): EP 792347 A1 970903 (Basic)
                             EP 792347 A1 990721
                             EP 792347 B1 051123
                             WO 1996011257 960418
APPLICATION (CC, No, Date): EP 95937405 951004; WO 95US12986 951004
PRIORITY (CC, No, Date): US 318409 941005; US 538544 951003
DESIGNATED STATES: AT; BE; CH; DE; DK; ES; FR; GB; GR; IE; IT; LI; LU; MC;
  NL; PT; SE
EXTENDED DESIGNATED STATES: SI
INTERNATIONAL PATENT CLASS (V7): C12N-001/00; C12N-001/12; C12N-001/20;
  G01N-033/531
NOTE:
  No A-document published by EPO
  Figure number on first page: 1
LANGUAGE (Publication,Procedural,Application): English; English; English
FULLTEXT AVAILABILITY:
Available Text Language Update Word Count
  CLAIMS B (English) 200547 613

Attachment

O 26107

30	92	7.2	1939	2	T18372	repeat organellar
31	91.5	7.2	399	2	G72253	RNA polymerase sig
32	91.5	7.2	472	2	A64320	PET112 homolog - M
33	91.5	7.2	508	2	T24622	hypothetical prote
34	91.5	7.2	615	2	S42797	rad 26 protein - f
35	91.5	7.2	1769	2	S53378	probable membrane
36	91.5	7.2	1847	2	E64477	replication factor
37	91	7.1	284	2	A45488	body-wall muscle t
38	91	7.1	359	2	AI2368	hypothetical prote
39	90.5	7.1	320	2	B97214	hypothetical prote
40	90.5	7.1	1017	2	D90550	vsaa-like (mycopla
41	90.5	7.1	1411	2	T18417	hypothetical prote
42	90.5	7.1	1417	2	T18418	hypothetical prote
43	90	7.0	404	1	S03849	ribonucleoprotein
44	89.5	7.0	280	2	A72046	conserved hypothet
45	89.5	7.0	280	2	F86578	CT671 hypothetical

#P1588

ALIGNMENTS

RESULT 1
D64718
conserved hypothetical protein HP1588 - Helicobacter pylori (strain 26695)
C;Species: Helicobacter pylori
C;Date: 09-Aug-1997 #sequence_revision 09-Aug-1997 #text_change 08-Oct-1999
C;Accession: D64718
R;Tomb, J.F.; White, O.; Kerlavage, A.R.; Clayton, R.A.; Sutton, G.G.; Fleischmann, R.D.
Peterson, S.; Loftus, B.; Richardson, D.; Dodson, R.; Khalak, H.G.; Glodek, A.; McKenne
son, J.D.; Kelley, J.M.; Cotton, M.D.; Weidman, J.M.; Fujii, C.; Bowman, C.; Watthey, L.
Nature 388, 539-547, 1997
A;Authors: Wallin, E.; Hayes, W.S.; Borodovsky, M.; Karpk, P.D.; Smith, H.O.; Fraser, C.
A;Title: The complete genome sequence of the gastric pathogen Helicobacter pylori.
A;Reference number: A64520; MUID:97394467; PMID:9252185
A;Accession: D64718
A;Status: preliminary; nucleic acid sequence not shown; translation not shown
A;Molecule type: DNA
A;Residues: 1-253 <TOM>
A;Cross-references: GB:AE000656; GB:AE000511; NID:g2314771; PIDN:AAD08627.1; PID:g231477

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Best Local Similarity 100.0%; Pred. No. 4e-88;
Matches 253; Conservative 0; Mismatches 0; Indels 0; Gaps 0;

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Qy          1 MAYKYDRDLEPLKQLESSDLLDLFEVLVPGKDGEKRHNEKLTSSIEYKRHGDDYAKYAER 60
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db          1 MAYKYDRDLEPLKQLESSDLLDLFEVLVPGKDGEKRHNEKLTSSIEYKRHGDDYAKYAER 60

Qy         61 IAEELQYYGSNSPASPIKGEGVLYKEILCDVCDKLKVNYNKKTETTLIEQNMLSKILERS 120
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db         61 IAEELQYYGSNSPASPIKGEGVLYKEILCDVCDKLKVNYNKKTETTLIEQNMLSKILERS 120

Qy        121 LEEMDDEEVKEMCDELSIKNTDNLNRQALSAATLTLPKMGGFKSYQLAVIVANAVAKTIL 180
              ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
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Qy        181 GRGLSLAGNQVLTRTLSPLTGPVGWIITGVWTAIDIAGPAYRVTIPACIVVATLRLKTQQ 240
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Qy        241 ANGDKKSLQIESI 253
              |||||||||||||
Db        241 ANGDKKSLQIESI 253
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RESULT 2
B71800
hypothetical protein jhp1494 - Helicobacter pylori (strain J99)
C;Species: Helicobacter pylori
A;Variety: strain J99
C;Date: 12-Feb-1999 #sequence_revision 12-Feb-1999 #text_change 08-Oct-1999
C;Accession: B71800
R;Alm, R.A.; Ling, L.S.L.; Moir, D.T.; King, B.L.; Brown, E.D.; Doig, P.C.; Smith, D.R.;

RESULT 1
AAW20486
ID AAW20486 standard; protein; 253 AA.
XX
AC AAW20486;
XX
DT 29-JUL-1997 (first entry)
XX
DE H. pylori cytoplasmic protein, 4095342.aa.
XX
KW Cytoplasmic; vaccine; prevention; treatment; infection; identification;
KW binding compound; bacterium; life cycle; activator; bacteria; inhibitor;
KW duodenal ulcer disease; chronic gastritis; diagnosis; envelope.
XX
OS Helicobacter pylori.
XX
PN WO9640893-A1.
XX
PD 19-DEC-1996.
XX
PF 06-JUN-1996; 96WO-US09122.
XX
PR 01-APR-1996; 96US-0630405.
PR 07-JUN-1995; 95US-0487032.
XX
PA (ASTR) ASTRA AB.
XX
PI Berglindh OT, Smith D, Mellgaerd BL;
XX
DR WPI; 1997-052306/05.
DR N-PSDB; AAT67811.
XX
PT Helicobacter pylori nucleic acid sequences and related
PT polypeptide(s) - useful for vaccines to treat or prevent H. pylori
PT infection, and to detect Helicobacter
XX
PS Claim 61; Page 651; 1481pp; English.
XX
CC The present sequence is a H. pylori cytoplasmic protein.
CC The protein may be used in a vaccine to prevent or treat H. pylori
CC infection or to identify H. pylori polypeptide binding compounds,
CC useful as potential H. pylori life cycle activators or inhibitors.
CC The genomic sequence of H. pylori (ATCC 55679) was determined from
CC overlapping contigs generated by mechanically shearing the bacterial
CC DNA. The sequences were analysed for ORF of at least 180 nucleotides,
CC and the predicted coding regions defined by computer evaluation. To
CC identify likely H. pylori antigens for vaccine development, the amino
CC acid sequences predicted from various ORF were analysed for significant
CC homology to other known or exported membrane proteins. Having identified
CC and determined the sequences of interest, particular regions can be
CC isolated from H. pylori by PCR amplification for recombinant polypeptide
CC production, e.g. in E. coli hosts.
XX
SQ Sequence 253 AA;

SEQID4

SEQID9

3

Query Match 99.3%; Score 1270; DB 18; Length 253;
Best Local Similarity 99.2%; Pred. No. 6e-118;
Matches 251; Conservative 1; Mismatches 1; Indels 0; Gaps 0;

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           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
Db       1 maykydrdleflkqlessdlldlfevlvfgkdgekrhnekltssieykrhgddyakyaer 60

Qy      61 IAEELQYYGSNSFASFIKGEGVLYKEILCDVCDKLKVNYNKKTETTLIEQNMLSKILERS 120
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
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Qy     121 LEEMDDEEVKEMCDELSIKNTDNLNRQALSAATLTLFKMGGFKSYQLAVIVANAVAKTIL 180
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Db     121 leemddeevkemcdelsikntdnlnrqalsaatltlfkmggfksyqlavivanavaktil 180

Qy     181 GRGLSLAGNQVLTRTLSPLTGPVGWIITGVWTAIDIAGPAYRVTIPACIVVATLRLKTQQ 240
           ||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||||
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Qy     241 ANGDKKSLQIESI 253
           || |||||||||:
Db     241 anedkkslqiesv 253
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Available online at www.sciencedirect.com
SCIENCE DIRECT
ELSEVIER
International Journal of Medical Microbiology 295 (2005) 343–353
IJMM
www.elsevier.de/ijmm

REVIEW

Helicobacter pylori vaccine development: Facing the challenge

Toni Aebischer, Andrea Schmitt, Anna K. Walduck, Thomas F. Meyer*

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Abstract

An effective vaccine would be a desirable way to control *Helicobacter pylori*-induced gastric disease. Initial studies in animal models demonstrated the feasibility of immunization and led to high hopes for a human vaccine. In the mouse model immunological approaches have to date not brought a satisfactory explanation for the mechanisms of protection against this largely luminal pathogen. Recently, transcriptome studies have identified new factors. It is now proposed that non-classical immune mediators may be the key to vaccine-induced protection.

Human trials of *H. pylori* vaccines are going ahead but although at least some formulations are clearly immunogenic, their effectiveness remains untested. The recent development of a human challenge model has now opened up new prospects for testing candidate vaccines and this will undoubtedly have a great impact in the near future. Future priorities for *H. pylori* vaccine development must be a better understanding of the protective mechanisms and the identification of biomarkers which can be used as reliable predictors of efficacy in humans. Despite some important advances in recent years, important issues must be resolved before an *H. pylori* vaccine will become a reality.

Keywords: *Helicobacter pylori*; Vaccine development; Mouse model; Human challenge model

Contents

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Introduction – Why a vaccine and for whom?

The current management of *Helicobacter pylori* infections relies on antibiotic therapy (Megraud, 2004; Moayyedi et al., 2000; Nakayama and Graham, 2004) (http://www.cdc.gov/ulcer/keytocure.htm). This strategy has a number of drawbacks including therapy failure due to emerging resistance, lack of patient compliance, side-effects of the antibiotics and high cost of treatment. Probably the most significant drawbacks of antibiotic therapy are its failure to prevent re-infection, and the increasing number of resistant strains; and these are the driving force to develop a vaccine against this worldwide infection (Ruggiero et al., 2003). An effective vaccine could improve two major aspects of disease management: as a novel therapy it could offer an improved treatment for the individual patient, and as a preventive measure a vaccine could eradicate the infection at the population level. It has been estimated that a 10-year campaign would eradicate the infection in developed countries even if the vaccine were only 50% effective. By contrast, continuous vaccination may be required in developing countries depending on prevalence of the infection (Rupnow et al., 1999, 2001). The target populations for therapeutic and prophylactic vaccines are different, as therapy is currently indicated for patients suffering from clinical symptoms, and this is normally restricted to adults. Serological surveys suggest that prevalence almost doubles between the age of seven and the late teens when it reaches steady levels (Malaty et al., 1999). Thus, prophylactic vaccination of both preschool- and school-age children may prove effective. Vaccination would therefore offer a cost-effective method of controlling *H. pylori* disease, providing therapy for the individual patient and the prospect of eradicating the infection on a population level.

What have we learned vaccinating mice against *H. pylori* infection?

Animal models of *H. pylori* infection have been instrumental in the development of strategies for immunization, and the establishment of the mouse model a decade ago has been particularly helpful because of its unsurpassed analytical power. Since the first encouraging studies which demonstrated that it was possible to reduce gastric *Helicobacter* colonization by vaccination with *H. pylori* antigen and adjuvant (Ferrero et al., 1995; Michetti et al., 1994), a wide variety of approaches including whole-cell vaccines, recombinant antigens (e.g. urease A/B subunits, CagA, VacA, NapA, catalase, or heat shock proteins) in combination with bacterial toxins or other adjuvants have been successfully tested. In addition, targeted mucosal delivery using live bacterial vaccine vectors such as *Salmonella* have also been successful. These studies have been comprehensively reviewed elsewhere (Blanchard et al., 2004; Del Giudice and Michetti, 2004; Sutton and Doidge, 2003).

In the mouse model, vaccination has been shown to be effective in both the therapeutic (Corthesy-Theulaz et al., 1995; Crabtree, 1998) and prophylactic case in adult mice (Garhart et al., 2002; Gomez-Duarte et al., 1998). More recently it was shown that neonatal mice can be protected by immunization. Intraperitoneal immunization with an alum adjuvanted vaccine was able to prevent transmission from infected mothers and also to eradicate already transmitted infection in the pups (Minoura et al., 2003). Similar results have also been reported using CFA and IFA adjuvanted vaccines (Eisenberg et al., 2003). As alum is already approved for use in children, this approach would appear to be feasible for clinical testing.

Despite encouraging results from the mouse model regarding protection three issues are always apparent: firstly immunization does not induce sterile immunity, but rather leads to (considerable) decreases in the number of bacteria, secondly the mechanisms by which the protective effects are mediated are unknown, and thirdly immunization induces an inflammatory infiltrate in the gastric mucosa which is histologically indistinguishable from that in infected animals (termed 'post-immunization gastritis'). Clearly these issues need to be resolved to enable the design of more effective vaccines. Accordingly, recent research has focused on clarifying the immune mechanism and the optimizing of vaccination strategies.

How is the protective immune response mediated?

Immunological studies focusing on the role of immune mediators have shed some light on this question and we now know that MHC II expression (Ermak et al., 1998; Pappo et al., 1999) and CD4 T cells are needed for protection. These CD4 T cells may be homing to the stomach by expressing $\alpha 4\beta 7^{+}$ integrins which enable them to bind to the muscosal addressins MadCAM-1 and VCAM-1. This may influence the outcome of immunization, since it was shown that blocking of these integrins prevented protection in a *H. felis* mouse model (Michetti et al., 2000). Given the importance of CD4 T cells, many studies investigated the protective immune response to *H. pylori* infection in view of the concept of the type 1 and 2 dichotomous CD4 T cell response, but to date this has been frustratingly uninformative. Recent work addressed the roles of the Th1-inducing cytokine IL-12 and TNF receptor in colonization and vaccination and showed that while colonization levels were affected, immunization was still possible (Panthel et al., 2003a).

Conflicting results have been obtained with IL-18-deficient mice: Panthel et al. (2003a) concluded that these mice can be vaccinated while Akhiani et al. (2004) reported no statistically significant effect. The most notable difference between these two studies is that despite using a similar vaccination strategy (*H. pylori* lysate plus CT), a different number of booster doses were administered (one and three, respectively). In addition, while the protective effect appeared to be not significant at 2 weeks post challenge (Akhiani et al., 2004), by 4 weeks the reduction of *H. pylori* colonization was statistically significant (Panthel et al., 2003a). In other studies on the role of interleukins in protection, Garhart et al. (2003) also showed not only that mice lacking both IL-4 and antibody were also able to mount a protective response, but that IL-5-deficient mice were also protected.

The data from these recent studies may now be added to that of previous studies and we are now forced to conclude that neither antibody, IL-4, IL-5, IL-13 (Aebischer et al., 2001; Garhart et al., 2003; Lucas et al., 2001), IL-12, TNF-α (Panthel et al., 2003a), nor IL-18 play a major role in vaccine-induced protection. While we now have an extensive list of what does *not* cause protection, there is an embarrassing lack of information on what might actually be responsible. Sutton (2001) has already suggested that gastric mucin probably plays an important role in vaccine-mediated protection, but experimental evidence has so far not been provided. In the absence of a role for an antigen-specific response via antibody, one might speculate on a role for 'innate' immune factors such as defensins, but here also further work is required. Inflammation clearly does have an effect on the survival of *H. pylori* in the stomach, and in IL-10-deficient mice for example *H. pylori* infection triggers a vigorous inflammatory response to infection and the pathogen is lost over time (Chen et al., 2001; Panthel et al., 2003a). It has therefore been proposed that *H. pylori* favors the induction of regulatory phenomena to limit inflammation and to allow long-term colonization (Blanchard et al., 2004). Blanchard et al. (2004) recently proposed a role for regulatory T cells (T reg) in *H. pylori* vaccination and suggested that the site of T cell activation influences protection. These authors also argue that infection with *H. pylori* leads to activation of T cells in the gastric mucosa but these T cells are unable to generate an effective response because they are suppressed by a population of T reg cells. They further speculate that in immunization, T cells become activated in the peripheral lymph nodes and the formation of T regs is not favored. The role of T regs in immunization, however, remains to be demonstrated. Furthermore, the relevance of these phenomena to immunization are unclear, as we have observed effective reduction in *H. pylori* burden in mice even before an inflammatory response can be detected at the histological level (Walduck et al., 2004). In addition, several groups have demonstrated that individual *H. pylori* proteins such as VacA have pharmacological effects that inhibit cell proliferation and possibly cytokine secretion by T cells after in vitro activation, and it has been proposed that this may contribute to the establishment of chronic infection (Gebert et al., 2003, 2004; Montecucco and de Bernard, 2003; Sundrud et al., 2004). In spite of these response-attenuating scenarios, it remains that vaccination is effective both prophylactically and therapeutically in animal models.

In an attempt to overcome the gap in knowledge between the obviously necessary activation of CD4 T cells and the reduction of *H. pylori* load, global analyses were initiated to shed new light on protection. Three different microarray studies (Mueller et al., 2003; Rahn et al., 2004; Walduck et al., 2004) investigated transcription profiles in immunized and non-immunized mice. The studies used different immunization and infection protocols, and also looked at gene expression time points ranging from immediately after challenge (Walduck et al., 2004), to several weeks (Rahn et al., 2004) and 22 months (Mueller et al., 2003), but nevertheless found to some extent similar genes regulated. Regulated genes in protected mice included T cell-specific genes (e.g. *Ly64, Slfn3*), MHC II genes and B cell-specific genes (e.g. *Ly57, CD40*). While Rahn et al. (2004) looked specifically for genes involved in inflammatory processes, the other two reports found that additional epithelial specific genes such as *Crpd* which is involved in innate defense, and adipocyte-specific genes (termed adipokines) (e.g. *Adn, Acrp30*) were up regulated in protected mice. Adipokines play a role in inflammatory immune responses (Trayhurn and Wood, 2004), and there is also evidence that e.g. the adipokine leptin influences T cell responses (Lord et al., 1998) and that T cells themselves produce leptin (Siegmund et al., 2004). The involvement of non-classical immune mediators may explain in part why immunological studies focusing on Th1/2 responses have not been informative so far (Lucas et al., 2001; Mohammadi et al., 1997).

In summary, transcriptome analyses have revealed new aspects to the process of protection, and it will be exciting and challenging to link these to T cell activation or clearance mechanisms, both of which may be downstream of these novel mediators. In this context much may also be learned form other models, such as arthritis and inflammatory bowel disease where adipokines may also be relevant. While the newly identified factors offer new possibilities, their role remains speculative and a great deal of work is required before we may benefit from this knowledge for vaccine design.

How can we optimize vaccination strategies?

Investigations on the effect of the route of immunization showed surprisingly that protective mechanisms against this mucosal infection can also be induced parenterally (Eaton et al., 1998; Guy et al., 1998). Others made efforts to improve mucosal delivery of vaccines by using new vehicles or increasing the efficacy of existing approaches. For example, Rizos et al. (2003) utilized constructs based on the *Escherichia coli* AIDA-I (*E. coli* adhesin involved in diffuse adherence) auto-transporter domain to display fragments of UreA on the surface of *Salmonella typhimurium* to improve the performance of *Salmonella*-based vaccination. When tested in BALB/c mice, surface exposure of a large UreA fragment and even a single, predicted T-cell epitope induced significant reductions in *H. pylori* colonization after a challenge infection, superior to cytoplasmic expression of UreA.

A number of novel approaches to delivery of *H. pylori* vaccine have been reported recently. Smythies et al. (2005) reported on *H. pylori* vaccination based on a modified polio virus vector where the capsid genes are replaced with *H. pylori* urease B. Poliovirus UreB replicons were co-administered with a recombinant vaccinia virus engineered to express polio virus capsid proteins, resulting in a vaccine which can only undergo one round of infection. Mice which are transgenic for the human poliovirus receptor (C57BL/6/DAB) are susceptible to infection with poliovirus via the systemic route. Replicon vaccination resulted in clearance of an established *H. pylori* infection in 73% of mice compared to 31% of vector-immunized controls. Furthermore, immunization prevented an infection from becoming established in 80% of immunized mice.

Bacterial ghosts (Gram-negative bacterial cell envelopes, devoid of cytoplasmic envelopes) have also been shown to have good adjuvant properties (Hoffelner and Haas, 2004). *H. pylori* ghosts induced protection in a mouse model without the use of an additional adjuvant although batch-to-batch variations were observed and improvements are therefore required before this approach could have practical applications (Panthel et al., 2003b). Sodium alginate microbeads have also been tested for controlled release of a model *H. pylori* vaccine (Leonard et al., 2004). Alginate beads are widely used for encapsulation of drugs, and the mild formulation conditions, and their reported muco-adhesive properties should make them ideal carriers for vaccine antigens. Recombinant urease encapsulated in alginate beads was administered to mice via the subcutaneous, nasal and oral routes. Unexpectedly, only subcutaneous delivery induced a significant antibody response and led to reductions in *H. pylori* colonization (as determined by urease test) indicating that this approach also needs further improvements (Leonard et al., 2004).

DNA vaccines are a potentially attractive approach to vaccination, and a genomic library approach has shown encouraging preliminary results in mice (Dzwonek et al., 2004). Two recent studies have investigated the adjuvant properties of CpG motifs in the context of DNA immunization. Interestingly, a prototype immunization construct encoding the UreB subunit which included CpG motifs (Hatzifoti et al., 2004) induced significant increases in the expression of IL-10 and beta-defensins in the gastric mucosa. In an approach that aimed to induce and modulate the immune response by triggering a specific Toll-like receptor (TLR), Sommer et al. (2004) immunized C57BL/6 mice with *H. pylori* lysate mixed with a synthetic CpG oligonucleotide targeted at TLR-9 (CpG oligonucleotide 1688). Immunization induced a Th1-biased immune response as expected, and immunized mice had 10-fold reduced levels of *H. pylori* in the gastric mucosa after challenge. Synthetic CpGs have recently been approved for human use as a therapy for genital warts (Garland, 2003), and so given the encouraging results from mice this approach might also be applicable for a human *H. pylori* vaccine. However, DNA vaccination studies in human volunteers have reported only suboptimal immune responses (Wang et al., 2004) and it appears that the barriers to DNA uptake may be more difficult to overcome in humans (Manoj et al., 2004).

To return to our original question regarding what we have learned from the mouse model, the data from animal models of *H. pylori* infection support the feasibility of both therapeutic and prophylactic vaccination, for neonates and adults. Furthermore, a variety of routes of application and adjuvants are effective. It is, however, clear that only a better understanding of the underlying immune mechanisms will make it possible to improve efficacy and to address the issue of post-immunization gastritis.

Can we translate what we have learned from the mouse model to humans?

A productive answer to this question will depend on the answers to some more focused questions relevant for vaccine design (see below).

Can candidate subunit vaccines be validated in mice?

We believe that the answer to this question is 'yes'. The mouse immune system faces the same problem as the human immune system, that is one of detecting a mostly luminal bacterium. This conclusion is supported by the observation that sera from both infected mice and

patients recognized by and large the same antigens from *H. pylori* (Bumann et al., 2002). Recently, a set of criteria has been proposed to identify potentially protective antigens, by using immunoproteomics data sets and the genome information of the two completely sequenced isolates (Alm et al., 1999; Tomb et al., 1997) (that probably describe >70% of the ORFs found in the species, M. Achtman, personal communication). The authors propose that vaccine antigens should be immunogenic in natural infections, belong to abundant protein species and in addition be specific and conserved amongst *H. pylori* to maximize protective coverage (Sabarth et al., 2002). All of the protein antigens that had been positively evaluated in mouse models of vaccination fulfilled the basic criteria of immunogenicity and abundance (Del Giudice et al., 2001; Sabarth et al., 2002), although some of them would have been rejected because of their widespread expression in other species. However, two novel protective antigens that fulfill all criteria were identified using this process (HP0410 and HP0231). The ever-increasing information on microbial genomes and a comprehensive list of more than 600 immunoreactive antigens described through proteomic approaches (Kimmel et al., 2000; Krah et al., 2004; Nilsson et al., 2000) now allow us to up-date and refine these criteria. At least three antigens, NapA, HP0410 (putative neuraminyl lactose-binding hemagglutinin homologue) and HP0231 (homologue of DsbA and DsbC, which have been described to oxidize protein thiols and have a potential role in the periplasmic folding of proteins in *E. coli*, (Bessette et al., 2001)) fulfill all criteria. Although CagA and VacA show allelic variation, these have been thoroughly characterized and can be included in the list of candidate antigens. We conclude that the number of available targets is not the current bottleneck in vaccine development.

What kind of adjuvant should be preferred based on the data from animal models?

As described above, a number of adjuvants and routes of immunizations have been tested, and these had similar efficacy in animal models. At this stage, from a practical and safety point of view, the most important consideration when designing human studies is therefore the selection of adjuvants or carriers which are licensed for human use. Because alum is licensed for human use a formulation with alum and the recombinant antigens CagA, VacA and NapA injected intramuscularly was tested in human volunteers (Malfertheiner et al., 2002). This vaccine was very immunogenic but its efficacy has still to be determined. Synthetic CpGs have recently been approved for use in humans (Garland, 2003), and based on the encouraging results in mouse models, this approach might also be applicable for a human *H. pylori* vaccine. We followed a similar strategy in developing a live vaccine by taking advantage of the licensed typhoid fever vaccine strain Ty21a, a chemically induced mutant strain derived from wild type *S. enterica* serovar Typhi. Ty21a was engineered to express *H. pylori* urease A and B subunits because the safety of this carrier in humans is well documented and clinical trials could be initiated (Bumann et al., 2001; Metzger et al., 2004).

Can the studies in animal models tell us what kind of immune response should be induced in humans?

The short answer to this question is 'no', and we have not yet learned how the immune reaction protects against *H. pylori*. However, it is worth considering the existing data as they may have practical implications for anti-*H. pylori* vaccination approaches. In mice, immunity can be induced by generating *H. pylori*-specific CD4 T cells, and CD8 T cells and antibodies are not essential. In humans, IgA deficiencies are relatively common and not correlated with more severe disease outcome due to *H. pylori* infection, and this would certainly be consistent with the idea that antibodies are dispensable for protection (Bogstedt et al., 1996). It is, however, precautious to conclude that either antibodies or CD8 T cells are irrelevant for protection. It is possible that the vaccines tested to date have not provided the right antigenic structures, and these structures could be subject to variation by *H. pylori* and therefore constitute moving targets. Conceivably, antibodies may inhibit colonization, and this is supported by the observation that pre-incubation of bacteria with urease-specific monoclonal antibodies suppressed infectivity (Czinn et al., 1993). With respect to CD8 cells, immunization experiments in β2M-deficient mice (Ermak et al., 1998) showed that a CD8 response was not essential to achieve a reduction in colonization, but a contribution to the immune response could not be ruled out. This may be particularly relevant when testing vaccines based on *H. pylori* antigens such as CagA or VacA that potentially enter the major MHC I presentation pathways in epithelial cells for example where they can be located in the cytoplasm (Segal et al., 1999) and in turn could be recognized by CD8 T cells.

Until the mechanisms behind protection have been clarified, it may be most appropriate at the moment to proceed with vaccine designs that are capable of triggering broad mucosal immune responses, i.e. mucosa homing CD4 T cells as well as CD8 T cells and antibodies. Potential immunization strategies could include prime-boost regimens with combinations of mucosal and parenteral routes of application (Lee et al., 1999), using antigen-encoding DNA vaccination and protein (Hatzifoti et al., 2004; Miyashita et al., 2002;

Todoroki et al., 2000) or live carriers such as *Salmonella* that can induce antibodies, CD4 and CD8 T cells specific for a vaccine antigen both systemically and mucosally.

Is a human vaccine feasible?

In the Jordan status report on vaccines in 2002 the anti-*H. pylori* vaccines had not progressed beyond phase 1 in clinical studies (http://www.niaid.nih.gov/dmid/vaccines/jordan20/). This is still valid. To date, only a handful of clinical vaccine trials have been conducted and these included only small cohorts of patients or non-infected volunteers to test the safety and immunogenicity of different vaccine formulations. Recombinant *H. pylori* proteins such as the virulence factors urease A/B subunits (Michetti et al., 1999), or CagA, NapA and VacA (Ruggiero et al., 2003), or chemically inactivated whole bacterial cells (Kotloff et al., 2001) were tested as vaccine antigens in combination with experimental adjuvants (e.g. wild type or mutants of the heat-labile enterotoxin of *E. coli* for mucosal application and alum for parenteral approaches). Vaccines with *H. pylori* urease subunits vectored in attenuated *S. enterica* serovars had also been evaluated (Angelakopoulos and Hohmann, 2000; Bumann et al., 2001; DiPetrillo et al., 1999). The outcome of these studies have been comprehensively reviewed and discussed (Blanchard et al., 2004; Del Giudice et al., 2001; Michetti and Svennerholm, 2003; Ruggiero et al., 2003), but the results so far support only a minimal conclusion, that is, *H. pylori* antigen-specific immune responses can be induced or, in the case of therapy, boosted by vaccination. A seminal clinical trial published in 1999 evaluated recombinant *H. pylori* urease in combination with wild-type heat-labile enterotoxin of *E. coli* as a therapeutic vaccine (Michetti et al., 1999). This is the only trial to report that vaccine therapy lowered *H. pylori* burdens in infected patients. While these results are encouraging, an effective measure of the protective effect is crucial to advance trials of a human vaccine.

How can we monitor protection in humans?

Ideally, therapeutic vaccination would eliminate the pathogen, and a prophylactic vaccine should prevent colonization. However, it is not clear that anti-*H. pylori* vaccines need to meet these endpoints in order to prevent the clinical manifestations of the infection. Nevertheless, *H. pylori* colonization is a conclusive parameter to determine the efficacy of vaccination. In the case of animal experiments, this is possible with high sensitivity by removing the infected stomach and determining the total bacterial burden in the organ. Obviously, in human trials this level of sensitivity cannot be achieved and the number of bacteria can only be estimated. To date, diagnostic methods (Cutler, 1997; Leodolter et al., 2001; Megraud et al., 2000), i.e. non-invasive approaches (urea breath tests, stool antigen tests), or invasive techniques such as gastric biopsies for quantitative culture, histological analysis or PCR have been used to determine *H. pylori* burden. None of these methods are satisfactory, however, and while non-invasive tests are often inaccurate and of low sensitivity, biopsies are prone to sampling errors, and the more sensitive approaches such as PCR lack standardization. Therefore, any improvement of these techniques to monitor vaccination outcome would be highly welcome.

While reduction in the bacterial load is certainly a valid parameter, from a clinical point of view the desired endpoint is prevention or amelioration of disease. Analysis of disease parameters should therefore be incorporated into clinical trials. Chronic *H. pylori* infection causes gastritis, alterations of the gastric pH (Ernst and Gold, 2000), affects gastrin and pepsinogen I levels in serum (Iijima et al., 2000; Levi et al., 1989), and local somatostatin expression (Sumii et al., 1994). These parameters are accessible and could therefore be exploited to assess effects of vaccination. Experience from the current eradication therapy regimes, however, indicate that changes in these parameters are slow or are complicated by other factors. Gastritis for example decreases only slowly after eradication (Iijima et al., 2000; Schenk et al., 2000; Tepes et al., 1999) and may depend on the host and additional clinical factors because improvement of gastritis can be expected in patients with gastric ulcer and duodenal ulcer, but probably not in patients with a tendency to develop non-ulcer dyspepsia (Talley, 1999). By contrast, increased serum gastrin levels appear to return to the normal range within 2 months after successful eradication also in dyspeptic patients (Gur et al., 1999), and these serum parameters could therefore be monitored. The human stomach is considered to be sterile, yet it has been observed that proton pump inhibitor (PPI)-treated *H. pylori*-infected patients display highly increased concentrations of non-*H. pylori* organisms in gastric juice samples compared to non-infected patients (Mowat et al., 2000). This indicates that monitoring the bacterial content of gastric juice in vaccine trials may be also of value.

An alternative approach is to identify novel correlates of protection. Ideally, such a marker will ultimately be accessible in the peripheral blood, e.g. via peripheral blood mononuclear cell stimulation. In an attempt to identify markers of *H. pylori*-induced disease, several groups have taken a global approach to define a molecular signature of *H. pylori* infection from patients with gastritis, diffuse, intestinal or mixed gastric cancer

(Boussioutas et al., 2003; Wen et al., 2004; Yasui et al., 2004). Just as molecular signatures could be used to predict disease outcome, it may thus be feasible to determine markers correlated with protection if a similar approach were incorporated into the design of a vaccination study and compared to the existing data sets.

While clinical parameters could be used to help assess the outcome of vaccination, there are still no validated biomarkers correlated with protection and this needs to be a focus of future research.

Should therapeutic or prophylactic vaccination be tested first?

Of course the answer to this question is that both applications will have to be tested eventually. The recent development of a human challenge model makes this choice realistic (Graham et al., 2004). As mentioned above, vaccine trials are complicated because the determination of protective effects is not straightforward. In the case of a therapeutic vaccine, this is even more complicated by the fact that *H. pylori* is very diverse and mutates frequently (Suerbaum and Achtman, 2004), and the vaccine antigen may not induce cognate responses against the patient's strain in all subjects. In addition, there is the imminent risk of worsening the disease because the host immune response contributes to pathology (El-Omar et al., 2000), and as discussed the beneficial effector mechanisms that reduce *H. pylori* burdens are unknown. Furthermore, regulatory phenomena established in chronic infections, exerted by regulatory T cells, or *H. pylori* factors that influence T cell responsiveness such as VacA (Boncristiano et al., 2003; Gebert et al., 2003; Montecucco and de Bernard, 2003; Sundrud et al., 2004), are likely to interfere with vaccine effects in therapeutic studies where already infected patients are vaccinated.

In the current phase of anti-*Helicobacter* vaccine development, the investigation of a human challenge model offers a remedy to some of the principle drawbacks of therapeutic studies to demonstrate the feasibility. Human challenge models have been instrumental in other bacterial diseases such as typhoid fever (Hornick et al., 1966), shigellosis (Tacket et al., 1992) and *Campylobacter jejuni* infections (Black et al., 1988) where bona fide animal models were also not available. With regard to the infection-induced pathology this also pertains to *H. pylori* infection. In a challenge model, the time and dose of infection can be controlled, the virulence traits of the infecting strain and its antibiogramme can be determined, target vaccine antigens can be verified, and, most important for the design of vaccination studies to obtain feasibility data, a relatively homogenous study cohort can be selected, and eradication of the pathogen to terminate the study can be predicted. A challenge model also allows the determination of acute reactions to infection which may be altered by vaccination and could help to define the much needed protection-related parameters. Controlled infection is not a novel approach in *Helicobacter* research as the self-infection by Marshall et al. (1985) and others (Morris et al., 1991) two decades ago has linked the bacterial infection with pathology. It is clear though that experimental infection with *H. pylori* can only be performed with adult volunteers able to give truly informed consent. It may be argued that adults are not a target population for a prophylactic vaccine and the approach is therefore questionable. However, in the history of vaccine development this argument affects more the choice of adjuvant than the principle mechanism of protection. We have recently tested the feasibility of the challenge model in a vaccination trial (T. Aebischer et al., in preparation). A small group of volunteers were vaccinated with recombinant Ty21a live vaccine and then challenged with the *H. pylori* strain developed by Graham et al. (2004). The initial results suggest that vaccination may indeed be feasible because diagnostic tests for *H. pylori* turned negative in a fraction of vaccinees after infection.

Conclusion – The perspectives for vaccination against *H. pylori*

A recent review on *H. pylori* vaccine development called for 'better vaccine formulations, better antigen preparation(s), better adjuvants, and better delivery systems' (Ruggiero et al., 2003). While we can only underline this statement, we would suggest that in addition much has to be learned about the protective mechanism, and the identification of biomarkers of protection should be a priority. For want of a clear hypothesis for the mechanism of protection, global analyses such as transcriptomics and proteomics to monitor host responses, and genome-scale mutational analyses of the pathogen (Kavermann et al., 2003) to define potential targets are still justified and necessary.

A more radical view is that the proof of principle of a human vaccine is lacking. It may be equally radical to propose that until we understand the mechanism of protection such proof should be sought with a prophylactic approach in adult volunteers since it can be controlled best. This approach has been critically evaluated (Michetti, 2004) in the perspective of developed countries where adequate treatment options are available. *H. pylori* infection is, however, a public health problem of mankind and is clearly related to poverty. The value of a vaccine, its pros and cons, risks and

benefits, obviously has to be discussed in a socio-economic context as well (Dawson, 2004; Grady, 2004). In order to substantiate this discussion, we believe that the feasibility of vaccination should at least be clarified.

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VALIDATION OF A MODIFIED KIRBY-BAUER DISK DIFFUSION METHOD FOR METRONIDAZOLE SUSCEPTIBILITY TESTING OF HELICOBACTER PYLORI.

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Triple therapy including metronidazole has been recommended as a first-line therapy with good eradication rates of H.pylori. Resistance in H.pylori to metronidazole has been reported worldwide. Various methods for testing H.pylori against metronidazole have been used including agar dilution, disk diffusion and the E-test but there has been little standardization of methods.

Methods: One hundred and six isolates of H.pylori from consecutive patients were tested for susceptibility to metronidazole by agar dilution (following NCCLS guidelines), E-test and disk diffusion (5ug disk). All three methods used Wilkens-Charlgren agar with 5% horse blood and were performed simultaneously from a 1 McFarland suspension of organisms in BHI broth.

Results/Conclusions:The agar dilution results confirmed the MIC susceptibility breakpoint to be <8mg/l. Using this breakpoint there was close agreement (93%) between E-test and agar dilution results. For susceptible strains, MICs by E-test were generally one twofold dilution lower. Agreement between disk diffusion zone diameter and MIC was 93% for agar dilution with breakpoints of >13mm and <8mg/l and 98% for E-test with breakpoints of >10mm and <8mg/l. The E-test discriminated better than agar dilution between susceptible and resistant strains and was simple to perform. The disk diffusion test is a reliable and cheap alternative to the E-test with susceptibility being a zone diameter >10mm with a 5ug disk. The prevalence of metronidazole resistance in this study was 38% by E-test.

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PHOTODYNAMIC THERAPY FOR THE TREATMENT OF HELICOBACTER IN THE FERRET STOMACH

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Antibiotic treatment for *H. pylori* is not entirely satisfactory. As we have already demonstrated that *H. pylori* can be killed by lethal photosensitisation in vitro, the purpose of this study was to determine the efficacy of this therapy, using the ferret model.

Explanted ferret stomach tied at the duodenum was filled with 2 mls of sensitiser, of varying concentrations, [Haematoporphyrin derivative, Phthalocyanine, Methylene blue (MB) or Toluidine blue] and the cardia tied. One hour later, the stomach was opened and the antrum divided into 4 strips 1 x 0.5cm. Each strip was halved, and one square exposed to light from the copper vapour laser at varying energy doses, whilst the other square was used as a control. All strips were placed in saline, homogenised and serially diluted (Miles and Misra) to obtain a viable count. One ferret was given oral Aminolaevulinic acid (ALA, 750mg/kg) 6 hours prior to sacrifice and treated as described above, to observe the effect of the endogenous sensitiser protoporphyrin IX (PPIX). A control stomach was processed to observe the effect of the laser light alone or no intervention.

MB at 50μg/ml combined with 50J/cm^2 laser light resulted in a 99% reduction in viable count, whilst increasing the MB concentration to 5000μg/ml resulted in complete eradication at the same energy dose. ALA treatment resulted in a 95% kill (160J/cm^2 energy dose) but none of the other sensitisers achieved significant kill. MB did exhibit some dark toxicity upon *Helicobacter mustelae* at 5000μg/ml but laser alone had no effect.

Exposure to low-power laser light kills *H. mustelae* sensitised by MB and PPIX, in the ferret stomach and raises the possibility of an alternative, non-antibiotic, method of eradication if it proves possible to treat all infected areas in vivo.

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PROGRESS TOWARDS A VACCINE AGAINST *HELICOBACTER PYLORI*

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Helicobacter pylori is one of the most prevalent infections of humankind and an important cause of gastrointestinal diseases worldwide. Because it is a chronic infection that persists lifelong while eliciting strong immune responses to multiple antigens, the feasibility of vaccination (particularly post-exposure vaccination) has been questioned. However, Czinn and Nedrud (*Vaccine* 1993; 11:637) and Chen et al. (*Lancet* 1992; i:1120) demonstrated that oral immunization with lysates of *H. felis* protected mice against hololog ous challenge, and Michetti et al. have demonstrated that recombinant *H. pylori* ureB complexed to hydroxylapatite is similarly effective (*Gastroenterology*, in press). We developed an efficient system for the expression of *H. pylori* urease apoenzyme in *E. coli*, and methods for its purification and stabilization as a mucosal vaccine. The recombinant apoenzyme was shown to retain both the ultrastructural integrity of native holoenzyme and reactivity with a protective monoclonal antibody. When administered into the oral cavity of outbred mice, 4 doses of 5 μg given at intervals of 1 week provided highly significant protection against subsequent challenge with *H. felis*, and doses of 25 μg were 100% protective. An adjuvant was required for protection, however, and this requirement could not be eliminated by administering high doses of antigen; cholera toxin and labile toxin of *E. coli* were effective adjuvants, but a derivative of muramyl dipeptide was not. Parenteral administration of urease induced a strong serum IgG response that was not protective. In contrast, immunization by mucosal routes elicited anti-urease serum, fecal and salivary IgA antibody responses that correlated with protection. The results indicate that prophylactic oral vaccination is feasible, that a subunit antigen (urease) is effective, and that secretory IgA mediates protection. Other studies will be reported on the precise role of cellular and humoral immunity in protection; on the identification of protective antigens other than urease; on post-exposure (therapeutic) immunization; on the vaccination of animal models susceptible to *H. pylori*; and on strategies for eliminating the requirement for adjuvants.

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ABSENCE OF EFFECT OF ERADICATION OF *HELICOBACTER PYLORI* ON GASTRIC ULCER RELAPSE, UNLIKELY TO DUODENAL ULCER.

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Purpose; Relapse rate of duodenal ulcer is markedly small after eradication of *Helicobacter pylori* (*Hp*). However, effect of the eradication on gastric ulcer relapse is still not clear. Here we examined whether the eradication of *Hp* is effective for prevention of gastric ulcer relapse or not in Japan. **Patients and methods;** The 28 patients with gastric ulcer and 11 patients with duodenal ulcers infected with *Hp* were assessed. Presence of *Hp* was evaluated by histology, culture, and CLO test. They were treated first with omeprazole 20 mg together with amoxicilin 1500 mg for two weeks and then, with omeprazole alone for 6 weeks. Endoscopy was performed before treatment, during treatment (at 4 and 8 weeks), and after treatment (every 2 or 3 months) to examine ulcer healing and relapse and presence of *Hp*. **Results;** Healing rate of gastric ulcer was 93% and that of duodenal ulcer was 100%. The eradication rate was 42.9% (12/28) in gastric ulcer patients and 45.5%(5/11) in duodenal ulcer patients. In gastric ulcer, cumulative remission rate was the same between the groups with successful eradication of *Hp* and those without (Fig. 1), while the rate was makedly higher when the eradication was succeeded in patients with duodenal ulcer (Fig. 2).

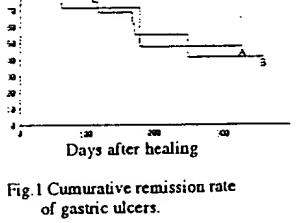

Fig.1 Cumurative remission rate of gastric ulcers.

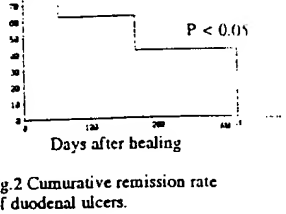

Fig.2 Cumurative remission rate of duodenal ulcers.

Conclusions; *Hp* may not have a crucial role in gastric ulcer relapse, unlikely to duodenal ulcer, at least in Japan.

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Chapter 17

GONORRHEA VACCINES

John W. Boslego and *Carolyn D. Deal*

ETIOLOGIC AGENT AND PATHOGENESIS

Description of the Agent

Neisseria gonorrhoeae is the etiologic agent of gonorrhea. The bacterium belongs to the family Neisseriaceae, which includes both pathogenic *(N. meningitidis)* and nonpathogenic *(N. sicca, N. subflava) Neisseria* (1). The clinical manifestations of gonorrhea, such as genital exudates, have been described for centuries, but it was not until 1879 that Albert Neisser first described the organism in urethral pus (2). *Neisseria gonorrhoeae* was first isolated in vitro in 1882.

Neisseria gonorrhoeae is a gram-negative diplococcus that grows on artificial medium at 37°C in a 5% CO_2 environment. The organism is oxidase-positive and ferments glucose, but not maltose, sucrose, or lactose.

Satisfactory treatment of clinical gonococcal infections was first achieved in the 1930s with sulfonamides (3). However, the organism quickly developed resistance to the drug, which has been a recurring theme. Penicillin was introduced for *N. gonorrhoeae* therapy in the 1940s and was highly successful (4). In the past 40 years, however, the organism has continued to evolve and manifest a variety of drug resistances (5). Penicillin can no longer be used in many regions of the world, including parts of the United States (5). Close surveillance of antimicrobial susceptibility patterns is necessary since the organism continues to develop resistance to the drugs used for its treatment.

Clinical Manifestations

Neisseria gonorrhoeae usually causes a local mucosal infection, but the spectrum of disease ranges from asymptomatic carriage to disseminated infection. The primary manifestations are urethritis in the male and cervicitis in the female. *Neisseria gonorrhoeae* infects other mucosal surfaces as well, resulting in conjunctivitis (neonatal and adult), pharyngitis, and proctitis.

Local extension of infection occurs in both sexes and is responsible for the major morbidity associated with gonorrhea. In men, the infection may extend to the epididymis, testes, or prostate. These unusual complications can result in sterility.

In the female, local extension of infection is more common, and more serious. Often acting in concert with other organisms, *N. gonorrhoeae* causes endometritis, salpingitis, peritonitis (pelvic inflammatory disease, PID), and perihepatitis. These infections can result in tubal scarring with infertility and subsequent ectopic pregnancy, tuboovarian abscess, and chronic pelvic pain. Estimates are that up to 45% of the women who contract a genital gonococcal infection will develop PID (6). Infertility rates after a single episodes of PID approach 15%, and after three episodes, 75% (6).

Disseminated gonococcal infection is manifested by papular or petechial skin lesions (usually on extremities), arthralgias, tenosynovitis, and oligoarthritis. Rarely, myocarditis, hepatitis, endocarditis, and meningitis occur. Strains causing disseminated infection are usually serum-resistant and of a particular auxotype (Arg^-,Ura^-,Hyx^-) (7). Patients who are genetically deficient in one of the terminal complement components are predisposed to repeated episodes of disseminated gonorrhea (8).

Epidemiology/Disease Burden

Gonorrhea is the most commonly reported infectious disease in the United States (6). Despite the relative ease of diagnosis and treat-

ment, and the extensive public health network for case identification and contact tracing, the epidemic continues.

In the United States, there are approximately 1 million cases reported each year (6). The highest attack rates are in young adults, ages 15 to 24 (6). It is estimated that the reported cases represent only about half of the actual cases. The number of cases peaked in the United States in 1975 and has plateaued and slowly declined since then (7).

Reliable estimates of disease rates worldwide are not available. Because many of the less-developed countries lack the resources for early diagnosis, treatment, and contact tracing, gonorrhea presents enoromous health problems in these regions. Epidemic infertility occurs in sub-Saharan Africa and is attributed to gonorrhea (6).

In the United States, the major morbidity and financial costs are related to PID. There are an estimated 400,000 cases of gonococcal PID each year. Considering the total number of cases of gonorrhea, the cost for evaluation and treatment (particularly of PID, infertility, and ectopic pregnancy) approaches $1 billion/year in the United States alone (6).

Pathogenesis

Neisseria gonorrhoeae is a uniquely human pathogen. The organism survives poorly outside the host unless artificially cultured. The disease is spread from person to person, usually by sexual contact with infected secretion. Once the bacteria are deposited on a mucosal surface, a series of events occurs that results in invasion of mucosal columnar cells and a host inflammatory response.

The stages of pathogenesis have been most closely studied in organ cultures of fallopian tubes and entail distant attachment of the organism to the host cell, close attachment and multiplication, ingestion by the epithelial cell, transportation through the cell in phagosomes, possible egestion onto the basement membrane, and, in rare instances, bloodstream invasion (9–11). The epithelial lining is markedly affected by gonococci, demonstrating loss of ciliary motility and extrusion of ciliated cells. The classic exudate consists mainly of host inflammatory cells, denuded epithelial cells, and gonococci.

The contribution of individual components of *N. gonorrhoeae* to each phase of pathogenesis is the subject of considerable study and will be discussed in more detail below. Recent developments in molecular biology have added substantially to our understanding of these events, but much remains to be learned.

HISTORY OF VACCINATION AGAINST GONORRHEA

Several gonococcal vaccines have been evaluated for efficacy in humans. The first effort was conducted by Greenberg et al. in Canada in the early 1970s (12). Greenberg et al. utilized three seed strains of type I (piliated) gonococci to prepare a killed, autolyzed vaccine. The vaccine was well tolerated and in the majority of volunteers stimulated an antibody response (bentonite flocculation, tissue culture neutralization) that was generally short-lived. Volunteers received three doses of vaccine, 1 week apart. In the study, conducted in a high-risk population, 62 volunteers were entered into a randomized, placebo-controlled trial. During a 12-month observation period, 10 of 33 vaccine recipients and 7 of 24 control recipients acquired gonorrhea, indicating no protective effect.

Brinton et al. later prepared purified pilus vaccines and tested them in human volunteers in a series of experimental challenge studies (13). The single-pilus vaccine was highly successful in preventing disease when the challenge strain was identical to the vaccine seed strain. The protection could be overcome by higher challenge inocula. Importantly, when a heterologous challenge strain was used, there was no apparent protection (C. Brinton, oral communication, 1982).

A field trail utilizing a purified single-pilus vaccine was conducted in high-risk U.S. military personnel in the Republic of Korea in 1983 (14). In this randomized double-blind, placebo-controlled trial, 3250 volunteers participated. Two doses of vaccine or placebo were administered 2 weeks apart. The observation period was 8 weeks. In male volunteers, 108 vaccine and 101 placebo recipients acquired gonorrhea 2 weeks or more after initial immunization. There was no vaccine protection despite the development of high levels of serum cross-reactive pilus antibody levels.

Lastly, a protein I vaccine was prepared and tested by E. W. Hook III in a human challenge model. The vaccine was well-tolerated and elicited a serum antibody response. This vaccine also afforded no protection against experimental gonorrhea (E. W. Hook III, oral communication, 1986).

VACCINE POTENTIAL OF IMPORTANT GONOCOCCAL ANTIGENS

Pilin or Peptides

Pili, filamentous projections from the cell, are one of the major surface antigens of the gonococcus. Each pilus is formed by the specific association of thousands of pilin protein subunits. This antigen displays both phase and antigenic variation. The organism can "switch" between a piliated and a nonpiliated state. Moreover, the subunits can display tremendous antigenic variation. The amino-terminal portion of the pilin sequence is highly conserved. Antigenic variation occurs predominantly in the carboxy-terminal region by insertions and deletions of two to four amino acid residues as well as single amino acid changes (15). The conserved amino-terminal sequence of the gonococcal pilin is homologous to that of other bacterial pilins including those from *N. meningitides* (16), *Pseudomonas aeruginosa* (17), *Moraxella nonliquefaciens* (18), and *Bacteroides nodosus* (19). All these sequences begin with the unusual *N*-methylphenylalanine residue.

Several studies have implicated pili as a virulence factor and as a potential vaccine candidate. Initial studies by Kellogg et al. reported that virulence in a human challenge model was correlated with certain colony morphologies (T1, T2) that were later discovered to represent piliated phenotypes (20, 21). Electron microscopic studies have implicated pili as mediating attachment of the gonococcus to the microvilli of nonciliate columnar epithelial cells (22). The pilus may somehow enable the organism to overcome the normal repulsive electrostatic barrier to attachment between the gonococcus and the host cell (23). Alternatively, a specific receptor–ligand interaction is possible, though no specific eukaryotic receptor for the pilus has yet been identified. Certainly, the presence of pili enhances attachment to a variety of eukaryotic cells. In addition to adherence, piliated gonococci have also been reported to be more resistant to phagocytosis than nonpiliated organisms (24).

Brinton et al. demonstrated that immunization of human volunteers with purified pilin resulted in the generation of antibody that was protective against challenge with the homologous strain (13, 25). In another human infection study, the expression of the pili was again correlated with virulence of the gonococcus, with piliated organisms causing disease and nonpiliated ones being avirulent (26). More importantly, this study demonstrated the in vivo antigenic variability of pilin. Disease isolates expressed numerous and different pilin types compared with pilin of the input strain. It is this capacity for antigenic variation in vivo that may allow the organism to circumvent a pilus type–specific antibody response.

The molecular mechanisms for antigenic variation have been studied by several laboratories (27–29). The variation is mediated by silent copies of pilin genes of differing antigenic types and locations on the gonococcal chromosome. Gene conversion of these copies into the expression site gives rise to expressed pilin of different antigenic types (30). One method of phase variation is deletion of the pilin gene at the expression site, which results in a nonpiliated phenotype, which can sometimes revert back to a piliated form (29). Recent reports indicate that another mechanism may also be involved, and that is DNA transformation of pilin genes between organisms (31). Thus, the gonococcus displays several sophisticated mechanisms for variation of this major surface antigen.

Selective regions of the pilin sequence, presented to the immune system as synthetic peptides, may make effective immunogens (32). Hopefully, a polypeptide representing only a sequence-conserved region may allow the resultant antibodies to effectively block all gonococci, but that is still speculative at this point. Studies to determine the x-ray crystallographic structure of the pilin molecule should facilitate the identification of exposed regions, which could be effective in this regard (33).

Pili, then, appear to be a major virulence factor for the gonococcus. The concept of a vaccine composed of pilin, or some portion thereof, is of considerable interest, but optimism for its success is dampened by the degree of pilin antigenic diversity and the fact that the human immune response appears directed primarily against the variable portion (14).

Nonpilin Adhesins

In addition to pilin, Muir et al. have reported the presence of other proteins associated with the pilus fiber (34). These proteins copurify with pilin and may possibly be incorporated into the pilus supramolecular structure. It is suggested that these proteins may be analogous to those seen in uropathogenic *Escherichia coli*, in which proteins incorpo-

rated at the tip of the pilus mediate adhesion to carbohydrate receptors present in the urinary tract (35).

Other studies indicate the possibility of pilin-independent adhesins that may be present on gonococci and mediate binding to eukaryotic cells. Both piliated and nonpiliated gonococci bind to carbohydrate-containing glycolipids (gangliotetraosylceramide and gangliotriaosylceramide) that have been isolated from eukaryotic cells (36). If this function is biologically significant and common among gonococcal strains, then an adhesin protein, or a peptide corresponding to the binding domain, would make a feasible vaccine candidate. However, the role of these proteins in the pathogenic process is unclear at present, and little is known about their potential immunogenicity.

Outer Membrane Proteins

There are three predominant outer membrane proteins (proteins I, II, III) in the gonococcal membrane. The role of these proteins in the pathogenesis of disease is still speculative. Proteins I and II are antigenically variable, while protein III appears to be identical in all strains.

Protein I

Protein I accounts for the majority of protein in the outer membrane and is designated the major outer membrane protein (37). It is found in all gonococci and varies in molecular mass (32–37 kDa) among strains (9). Protein I is believed to function as the porin protein by forming hydrophilic channels through the outer membrane (38). At least a portion of the protein is surface-exposed (39).

The antigenic variability of protein I provides a useful mechanism to classify gonococcal strains. A single strain expresses only one protein I, which remains antigenically stable (9). There are two major subclasses of protein I: protein IA and protein IB. The amino acid sequence of representative strains of each subclass is now known (40, 41). Each subclass represents a family of structurally different, but similar, protein I's. Protein IAs are generally of lower molecular weight. While the amount of protein I that is surface-exposed tends to differ among strains, protein IAs have a smaller surface-exposed portion than protein IBs (42). The surface-exposed determinants form the basis of the current serologic classification schemes. A commonly used system employs six protein IA and six protein IB monoclonal antibodies (43). On the basis of its reaction pattern to this panel of monoclonal antibodies in a coagglutination assay, a strain can be classified into a serovar. To date, 24 protein IA and 32 protein IB serovars are recognized worldwide. In any given region, however, the overwhelming majority of strains are represented by far fewer serovars.

There are functional correlations between protein I subclass/serovar and characteristics of the organism and/or expression of disease. Protein IAs are associated with disseminated gonococcal infection and resistance to killing by normal human serum (44). Protein IBs are more closely associated with antibiotic resistances (45) and with local mucosal disease (46). However, these associations are far from absolute, and there is considerable overlap. Nevertheless, this typing scheme has been of considerable value in outbreak investigations and in epidemiologic studies of disease transmission (47–49).

Although the role of protein I in the pathogenicity of human infection is not established, there are several lines of evidence that encourage its further investigation as a vaccine candidate.

Protein I is essential for organism survival. It is surface-exposed and invariant in a given strain. There is considerable structural and antigenic similarity among strains in each subclass. It appears to interact at the eukaryotic cell membrane and may trigger endocytosis of the organism by the host mucosal cell (9).

Protein I is immunogenic in humans during the course of a natural infection. Patients develop protein I antibodies in their local secretions and serum as a consequence of infection (50–53). These antibodies exhibit both opsonic and bactericidal properties (54, 55). Moreover, many of the protein I monoclonal antibodies activate complement and lyse the organism (42, 55).

Clinical studies also support the concept that protein I antibodies may protect against infection. Buchanan et al. demonstrated that recurrent episodes of acute gonococcal salpingitis were not caused by gonococci of the same protein I type (56). More recently, Plummer et al. showed that female genital infection with a given serovar appeared to provide protection against a subsequent infection with the same serovar (57).

To date, one protein I vaccine was unsuccessful in a male gonococcal urethritis infection trial, as described previously. While dis-

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appointing, this trial should not impede the investigation of alternative protein I vaccines. Manufacturing a vaccine composed of a mixture of protein I serovars is one approach, but a quicker avenue for vaccine development lies in the identification of a conserved epitope(s) that is surface-exposed and the target of functional antibody activity.

The current or imminent availability of protein I sequence data, structural information on surface-exposed epitopes, and methods for gene cloning and expression will greatly enhance our understanding of this important antigen (40, 41, 58).

Protein II

Protein II is a 24- to 30-kDa heat-modifiable outer membrane protein (9). Protein II actually describes a family of related proteins (protein IIs) that manifest tremendous intrastrain and interstrain variations (59). The protein is at least partially surface-exposed. When expressed, protein II constitutes a high percentage of the outer membrane (60).

Protein II expression is associated, at least in part, with the ability of the organism to develop opaque colony types when grown on culture media and viewed under a dissecting microscope (61).

The antigenic variation of protein II has been the subject of considerable study. A single strain may simultaneously express more than one antigenically different protein IIs and switch from a protein II–positive to protein II–negative phenotype, or from one protein II type to another at a high frequency (59, 62).

Protein II appears to bestow increased adherence properties to gonococci (63). Protein II expression is associated with greater gonococci–gonococci adhesion, as well as increased adhesion of gonococci to human epithelial cells, conjunctival cells, and neutrophils (64). It is possible that different protein IIs mediate adhesion to different cell types.

The role of protein II in human infection in unknown. Clinical studies have shown that opaque variants are more commonly recovered from mucosal gonococcal infections, whereas transparent variants are more often recovered from asymptomatic or disseminated infections (65). Protein IIs are immunogenic in natural infection. Both males and females develop antibodies, but their antibacterial properties are not known (50, 66). Antigenic variation of protein IIs also occurs in vivo. In an experimental infection study, a broad array of protein II variants appeared during the course of infection after a predominately protein II–negative phenotype was instilled intraurethrally (67).

Protein II is currently not considered an attactive vaccine candidate. Although it is surface-exposed and quantitatively significant in protein II–positive organisms, the bacteria can survive and grow in its absence. Even though a pathogenic relationship is suggested by its adherence properties, too little is currently known to establish a defined role in human infection. Lastly, the phenomenal array of antigenically distinct protein IIs would make it a very difficult antigen to incorporate into a vaccine.

Protein III

Protein III is a 30- to 31-kDa outer membrane protein (9). It is surface-exposed and present in all strains of gonococci (68). Moreover, an analogous protein, designated class 4, also exists in *N. meningitidis* (69).

Protein III is closely associated with protein I in the bacterial membrane, but its function in bacterial physiology or in the pathogenesis of disease is not known (69). There is no evidence to support its role as a porin protein or as a cofactor for protein I in this capacity.

In stark contrast to other gonococcal surface antigens, there is no evidence for structural or antigenic variation in protein III (70). All avaliable studies suggest it is invariant among gonococcal strains. Moreover, protein III shares remarkable sequence and structural similarity to the Omp A proteins of *Enterobacteriaceae*, particularly *E. coli* (71). This homology is especially marked in the carboxy portion of the molecule (69).

Despite its surface location, protein III is poorly immunogenic in humans during natural infection (50, 66). Patients demonstrate either no response or low levels of antibody.

The most fascinating aspect of protein III is its apparent capacity to induce and/or bind to antibodies that block the bactericidal activity of antibodies to other surface antigens (protein I, lipopolysaccharide) (72, 73). There is experimental evidence that protein III antibodies (IgG) fix complement, but the resultant membrane attack complex is either defective or incapable of fully inserting into the cell membrane to cause bacterioloysis (74, 75).

Due to its antigenic similarity to Omp A proteins, patients may develop protein III antibodies as a result of colonization/infection

by *Enterobacteriaceae*. These cross-reactive protein III antibodies may then shield the gonococcus from the bactericidal activity of other antibodies. Protein III might then exist on the gonococcus as a mechanism for its own protection. If this scenario proves valid, protein III might be deleterious if incorporated into a vaccine preparation.

Considerable work is in progess to resolve these important issues. The recent construction of a protein III–deficient gonococcal strain will significantly aid in this evaluation (69).

Lipopolysaccharide

Lipopolysaccharide (LPS) is a major constituent of gram-negative outer membranes and is known to serve several important biological and pathogenic functions. In addition, various LPS epitopes, distinguishable by monoclonal antibodies, are expressed on different gonococcal strains or on the same strain at different times, resulting in LPS antigenic variation.

Phenol-extracted LPS has been shown to mediate most of the toxic damage that occurs during infection of human fallopian tubes (76). The LPS is a target for bactericidal antibodies and regulates complement activation on the bacterial cell surface (77, 78). The presence or absence of certain LPS epitopes may be involved in the determination of serum-sensitive or serum-resistant phenotypes (79).

The structure of gonococcal LPS is similar to that of enteric bacteria in that both have a lipid A fatty acid chain embedded into the cell wall, and a core oligosaccharide linked to three 3-deoxymanno-2-ketooctulosonic acid (KDO) moieties. The gonococcus differs from enteric bacteria, however, in that it lacks an *O* side chain of strain-specific polysaccharide residues (80). Silver staining of periodate-oxidized LPS and rapid isolation methods have enabled the determination of LPS molecular masses ranging from 3.2 to 7 kDa among strains (81). The development of LPS monoclonal antibodies has allowed the immunochemical characterization of specific LPS components (79). Recently the structural determination for the oligosaccharide portion of the gonococcal LPS has been proposed (82). Studies by Mandell et al. have shown that particular gonococcal LPSs have carbohydrate structures that are analogous to human erythrocyte glycolipids, and that these two structures cross-react immunologically (83).

The antigenic variation of gonococcal LPS structures was seen on passage in vitro (81). This variability was also demonstrated in vivo during a human challenge study in which the strains isolated from the infected patient expressed different LPS antigenic types from the challenge strain (84).

An LPS-based vaccine would necessitate the detoxification of the endotoxic-producing properties of LPS. In addition, given the antigenic diversity of the LPS, a constant oligosaccharide portion or a type correlated with virulence would have to be identified. The immunogenicity of this molecule in humans and its apparent role in pathogenesis designate it as another attractive vaccine candidate (77).

H.8 Epitope

An epitope contained on two different neisserial lipoproteins that binds to a specific monoclonal antibody is called H.8. The epitope itself appears to be conserved and stable (85). Following its identification in 1984, H.8 gained wide attention because of its presence on pathogenic *Neisseria (N. gonorrhoeae, N. meningitidis)*, but absence on commensal *Neisseria* (85).

The two lipoproteins that contain the H.8 epitope are the lipid-modified azurin (Laz) and the H.8 outer membrane protein (Lip) (86).

The lipid-modified azurin is present in both pathogenic and commensal *Neisseria* (86). It is not reactive with the H.8 monoclonal antibody on Western blots. Like other azurin proteins, this lipid-modified azurin may function in electron transport during bacterial respiration. Its role, if any, in pathogenesis is not known.

The H.8 outer membrane protein is also a lipoprotein, but it is present on pathogenic *Neisseria* only. Its apparent molecular mass varies from 22 to 30 kDa among strains (87). This protein is alanine- and proline-rich and does not stain with Coomassie blue after sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE). The H.8 outer membrane lipoprotein has been extracted and purified from neisserial organisms (87, 88), and the gene has been cloned and sequenced (89). The protein consists of a repeating heptapeptide. Differences in the number of repeating units may account for the difference in apparent molecular weight among strains. The function of Lip in the outer membrane is unknown, but it is believed to serve in a structural role.

The H.8 epitope appears to be surface-ex-

posed on viable gonococci, but electron microscopy studies with gold-labeled monoclonal antibodies have provided conflicting results (85, 86, 90).

Patients develop serum antibodies to the H.8 epitope after local genital infections, salpingitis, or disseminated gonorrhea (50, 51, 91). One patient was found to have H.8 antibody in seminal plasma (50). However, H.8 antibodies have also been found in the sera of patients without a prior history of gonorrhea. One study showed that the acquisition of *N. meningitidis* throat carriage can result in the development of H.8 antibody in the serum (50). In several studies, patients developed local gonoccal infections despite the presence of H.8 antibody in the serum (50, 66).

Most monoclonal antibodies specific for H.8 lack bactericidal activity and fail to protect against meningococcal infection in animal models (92). However, one H.8 monoclonal antibody has been shown to be bactericidal and opsonic for some gonococci (86). Affinity-purified human antibodies specific for H.8 were found to lack bactericidal activity for meningococci (93).

The value of the H.8 epitope as a gonococcal vaccine candidate is unclear. The apparent surface-exposure, stability, and association with pathogenic *Neisseria* make it worthy of pursuit; however, the lack of a known role in pathogenesis and the inability of serum antibody to prevent infection weigh against it.

IgA Protease

Immunoglobulin A represents a major host defense system against microbial pathogens that come in contact with mucosal surfaces (94). However, many bacteria, such as *N. gonorrhoeae*, *N. meningitidis*, *H. influenzae*, and *Streptococcus pneumoniae*, are known to produce a protease that cleaves human IgA1 at the hinge region (94). These IgA1 proteases are thought to act as a mechanism for bacteria to evade specific mucosal immunity. The construction of IgA1 protease-negative mutants of *N. gonorrhoeae* provides a valuable tool to investigate the significance of this enzyme in the pathogenesis of gonorrhea (95).

The gene encoding the gonococcal IgA1 protease has been cloned. Significant homology with IgA1 protease genes of other species was also found (96). Patients with local gonococcal infections, salpingitis, and disseminated gonorrhea infrequently produce antibody to IgA1 protease in their sera (97). Patients with meningococcal disease or meningococcal carriage more commonly produce specific antibody to the enzyme (97). This antibody is cross-reactive with IgA1 protease from *N. gonorrhoeae* and inhibits the protease activity of the enzyme (97).

The IgA protease presents an attactive vaccine candidate if its biological role in disease is validated. As part of a multicomponent vaccine, antibody raised to this enzyme may well allow IgAs of other specificities to be more efficacious in attacking the organism.

Major Iron-Regulated Protein

Neisseria gonorrhoeae expresses several proteins under iron-limited conditions (98). These proteins may be involved in iron uptake by the bacteria and therefore may have a function in pathogenesis. In contrast to many gram-negative bacteria that produce soluble siderophores, the gonococcus must obtain iron directly from specific iron-binding proteins (lactoferrin and transferrin) of the host (99).

The major iron-regulated protein (MIRP) is a 37-kDa protein and appears to be common among all gonococci and meningococci (100). Morse et al. have recently described its purification and characterization (101). Antibodies to MIRP have been detected in patients with disease, indicating that it is expressed in vivo (102). This protein is reported to bind iron from transferrin (101).

The apparent immunogenicity and conserved nature of the MIRP makes it an attractive vaccine candidate. Other proteins that mediate iron acquisition also deserve attention. Interference with iron utilization could potentially alter the course of disease.

Other Gonococcal Antigens

Anaerobic or aerobic growth conditions induce strains to selectively express a variety of membrane proteins. Other conditions of environmental stress result in the expression of another class of proteins called *stress proteins*. The role of these proteins in organism survival or pathogenesis is not known. Yet it is interesting to note the presence of antibodies to an anerobically induced protein in women with PID, thus suggesting its immunogenicity and expression in vivo (103).

Gonococcal outer membrane protein–macromolecular complex is a surface-exposed homopolymer. It is antigenically conserved and constitutes about 10% of the outer membrane protein (104). Antibodies raised to this antigen in animals exhibit bactericidal activ-

ity (104). Little is known of its function or role in disease.

Numerous other proteins, as yet unnamed and uncharacterized, appear in SDS-PAGE of gonococcal lysates. Some of these proteins appear to elicit a serum antibody response to infection (50, 51). Work is just beginning to evaluate their importance.

GENERATION OF MUCOSAL IMMUNITY

The presence and duration of genital mucosal immunity is an issue of critical importance for the development of a gonococcal vaccine. A parenteral injection with purified pili can induce in the genital tract antibodies that inhibit attachment of gonococci to epithelial cells in vitro (25, 105). In addition, studies in women have demonstrated high concentrations of antigonococcal IgA following infection (106, 107).

Humans may possess a "common" mucosal immune system in which antigenic presentation at one mucosal surface can lead to trafficking of secretory IgA–producing cells to other mucosal locations (108). Perhaps oral or intestinal immunization will lead to a genital immune response, since secretory IgA (S-IgA) is the major immunoglobulin of mucosal surfaces and is produced by plasma cells in the lamina propria (109). Exposure of an antigen to the Peyer's patches in the intestine can stimulate T cells of various types, and precursor IgA B cells, resulting in an IgA response at distant mucosal sites, such as the genital tract (108).

Examples of this phenomenon have been seen in oral immunization with *Streptococcus mutans* that resulted in significant S-IgA response in the saliva and tears (110). For *Neisseria*, a protein I vaccine administered in the intestines of rats induced antibody in lymphoid organs (111).

VACCINE PROSPECTS AND RESEARCH DIRECTIONS

Gonorrhea continues as an epidemic disease with serious complications, especially for the female. The current strategy of selective screening, contact tracing, education, and improved clinical care has served only to contain the epidemic in developed countries. Lacking resources for implementation of these strategies, less-developed countries have even greater problems.

Despite effective antibiotic treatment, the pool of minimally symptomatic and asymptomatic carriers serves as a reservoir for transmission. Upon clinical presentation, many women already have PID. While the infection is treatable, the sequelae remain. A strategy to prevent infection will have widespread impact, and a vaccine is highly desirable.

The evolution of antibiotic resistance in the gonococcus hastens the quest. Parenteral antibiotic injections for treatment are again needed in many regions, and close vigilance of susceptibility patterns is mandated.

The development of an effective gonococcal vaccine will be a formidable task. Many individuals acquire gonococcal infections repeatedly, implying the absence of or short-lived immunity. In the preantibiotic era, untreated infections persisted for months, implying markedly delayed, if any, disease-induced immunity. In part, this may be due to the usual confinement of infection to the local mucosa. Antigen processing at the genital mucosal surface is problematic. Moreover, antibody presence, particularly at the critical time of organism arrival, would be expected to be quantitatively minimal.

Gonococci also seemed equipped with an outstanding arsenal to avoid the host's immune defenses. The capacity for on–off switching that controls antigen expression (pili, protein II), combined with the capacity for antigenic variability (pili, protein II, LPS) is staggering. Another antigen, protein III, may actually serve as a target for cross-reactive antibodies and block access of specific functional antibodies. The suggestion of a shared epitope between LPS and the erythrocyte membrane raises the possibility of antigenic mimicry, a mechanism to avoid antibody production. Finally IgA protease may serve to destroy any secretory antibodies that arrive for defense.

Add to this the lack of protection of whole-cell and purified pili vaccines in human field trials, and the skepticism expressed by some concerning the development of an effective vaccine appears well founded.

But there are reasons for persistence and optimism. Older studies demonstrated a correlation between a prior gonococcal infection and relative resistance to urethral challenge (112). Two studies now suggest the development of protein I–type or serovar-specific immunity after female genital infection. Most encouragingly, there was development of ho-

mologous protection to urethral challenge after parenteral immunization with purified pili.

The ideal vaccine would be one that prevents colonization and local infection. Given the uniquely human reservoir of *N. gonorrhoeae*, such a vaccine may eventually lead to eradication of the organism. If prevention of local infection is not possible, an alternative approach would be a vaccine that limits extension of infection and prevents PID. Such a vaccine would have a profound impact on the morbidity of gonorrhea.

The evaluation of vaccine candidates must proceed cautiously. Should a vaccine allow or predispose to asymptomatic carriage, it might have a deleterious public health effect. Increasing the reservoir of carriers would predictably result in a higher incidence of gonococcal disease, including PID, among the unimmunized.

A successful vaccine will likely be one that induces antibodies that simultaneously attack the organism at several stages of pathogenesis. One might envision the success of vaccine-induced antibodies that allow human IgA survival, promote opsonization and bacteriolysis, and yet block organism adherence, iron usage, and eukaryotic membrane integration.

Appropriate models to evaluate pathogenesis and immunity are a problematic area. Numerous animal models have been employed, but only the chimpanzee urethritis model bears any resemblance to human disease. The availability, difficulty, and cost of these animals have curtailed the use of this model. Other animal models such as the guinea pig chamber and chick embryo are also in use, but their relevance to human disease is unknown.

Tissue and organ cultures are in selective use. Much about pathogenic mechanisms has been learned from the fallopian tube system.

Ultimately, the human will serve for the final validation of a successful vaccine. Human challenge studies have been of considerable value in the past for investigations on pathogenesis and for vaccine testing. The model is safe, and the experimental infection closely resembles naturally acquired disease. More studies are needed in this model to understand the contribution of specific gonococcal components to human disease and to investigate the human immune response. Ethical considerations limit this model to male urethritis only, so the evaluation of vaccines that might prevent pelvic inflammatory disease will have to take place in the field.

Ongoing and needed investigations that have the greatest bearing on vaccine development lie in the following three areas:

1. Studies on the molecular basis of pathogenesis that increase our understanding of the critical steps leading from organism exposure to infection are essential. Particular emphasis should be placed on the contribution of individual antigens to this process. Identification of gonococci–host cell interactions, as well as host cell receptors, are a critical phase of these studies.
2. Studies on the extent of antigenic variation, the mechanisms for its control, and the contribution of the host environment and immune response will allow a realistic assessment of potential vaccine candidates. The discovery of common, stable, and functional epitopes in this sea of variability holds the brightest hope for an effective vaccine.
3. Studies on the local immune response in the human genital tract, methods to enhance it, and the value of specific antibodies in protection are vital to our continued progress. Investigations should continue on local immunization procedures.

Many new technologies are now widely available to aid in these investigations. Gene cloning, DNA and protein sequencing, protein structure studies, synthetic peptides, new adjuvants, epitope mapping, the polymerase chain reaction, and the construction of defined mutants can now be brought to bear. It will likely require the close collaboration of many groups with diverse skills in order for us to be successful in the quest for an effective gonorrhea vaccine.

Note: The views of the authors do not purport to reflect the position of the Department of the Army or the Department of Defense.

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29 New Technologies for Making Vaccines

The development of new techniques in molecular genetics has expanded the number of approaches that can be used for making vaccines. In some cases, established vaccines can be improved or their supply increased. In other cases, new vaccines can be developed that have not been feasible through the application of old technologies. In this regard, it is worth considering first the broad categories into which vaccines can be divided. "Live" vaccines are defined by the ability of the vaccine strain, i.e., of the virus, to replicate within the human host. Conversely, "killed" vaccines ("non-live" may be more accurate, even though most scientists use the term "killed") are unable to replicate or infect the host. Table 29–1 summarizes the salient features of these two categories of vaccines.

Live Vaccines

Live vaccines are attenuated with respect to their ability to cause disease, meaning that they are less likely to cause clinical illness than the natural disease-causing agent. By virtue of their ability to undergo limited replication in the host, such vaccines, typically viruses, often induce cell-mediated (T cell) immunity in addition to antibody-mediated (B cell) immunity. As a result of such a broad spectrum of immunity as well as re-exposures to the virus which silently boost immunity, protection following a single inoculation with a live attenuated vaccine often lasts a lifetime. However, the ability of the live vaccine to replicate can be detrimental; being genetically plastic, a replicating virus can revert to a more pathogenic form and cause adverse reactions in a vaccinee or a contact of a vaccinee. Sufficient data must be obtained in animal studies as well as in clinical studies to rule out the possibility of reversion.

A number of strategies have been employed for developing live viral vaccines that are attenuated, as summarized in Table 29–2.

Several of these approaches were possible before the development of modern techniques in recombinant DNA (rDNA) technology which enable the manipulation of viruses on the molecular level. These classic approaches, which utilize routine techniques in cell culture, include attenuation in cell culture, selection for temperature-sensitive or cold-adapted viruses, isolation of closely related viruses from other species and selection for reassorted viruses from the progeny of an infection by two parental viruses. (These strategies are discussed in greater detail elsewhere with respect to particular vaccines.)

The ability to alter directly the structure of viruses on the molecular level is enabling scientists to design attenuated vaccines rather than forcing them to rely upon phenotypic selection and upon chance to provide the only mechanisms for viral change. Through techniques of viral genetics and DNA sequence analysis, it is possible to identify those regions in the viral genome where alteration can contribute to the attenuation of viral pathogenicity. This rDNA technol-

Table 29–1. General Characteristic of Vaccines

"Live" Vaccines
Attenuated with respect to pathogenicity
Cell-mediated immunity in addition to humoral immunity
Longer-lasting protection
Tendency to reactogenicity
Ability to revert
"Killed" Vaccines
Nonreplicating
Noninfectious
Lower reactogenicity
Need for boosters
High purity

Table 29–2. Strategies for the Development of Attenuated Live Viral Vaccines

"Classic" Approaches
Modified by passage in cell culture
Variant viruses from other species
Temperature-selected mutants
Reassorted genomes
"Molecular" Approaches
DNA modification mutants
Recombinant viruses

ogy allows such regions to be altered or deleted and introduced into the genome of a wild-type virus, thus leading to the production of an attenuated virus. This approach is presented in the schematic in Figure 29–1.

The salient feature of this approach is the deliberate construction of an attenuated virus that is unlikely to revert to a more pathogenic form. This construction is made possible by deleting a portion of a key region of the genome in such a way that reversion is ruled out. This approach first was applied successfully by Kit and coworkers to the attenuation of pseudorabies virus, thus leading to the creation of a safer vaccine for the prevention of a severe disease in pigs.[1] This is the first genetically altered live vaccine that was licensed for use in any species. A related approach is being taken for poliovirus and is applicable to other vaccines for humans.[2]

A second approach is the genetic alteration of a live virus to function as a vector, *i.e.*, carrier, for other genes. This approach enables the recombinant virus to function as a vaccine for two or more infectious agents in a single inoculation. This technology first was applied to vaccinia virus.[3, 4] Prior to this application, wild-type vaccinia virus had been used for the worldwide eradication of smallpox and is the prime example of a variant virus from another species used as a vaccine for humans. A region of the genome of vaccinia virus was identified as nonessential for viral replication by the general approach outlined in Figure 29–1. Within a plasmid containing this nonessential region, a gene encoding a surface protein of another pathogen was inserted (Fig. 29–2). This recombinant plasmid was introduced together with wild-type virus into cells in culture, resulting in the creation of a recombinant virus that carries the foreign gene.

For insertion into a virus vector, a gene is selected that encodes an immunogen, usually a surface protein, of a virus or a microbial parasite. In order for this strategy to be effective, the presentation of this immunogen during the course of viral replication should result in a protective immune response directed to the antigen and, therefore, the pathogen. Recombinant vaccinia viruses have been derived that express immunogens for hepatitis B virus, herpes simplex virus, influenza virus, rabies virus, Epstein-Barr virus and respiratory syncytial virus. Some of these recombinant viruses have shown promise in animal studies. A similar approach has been taken with respect to the genetic engineering of two human herpesviruses as viral vectors, herpes simplex virus[5] and varicella-zoster virus.[6]

Table 29–3 outlines several points that are important to the safety and efficacy of such live recombinant vaccines. A nonessential (i.e., not required for viral replication) region for the insertion of a foreign gene often can be used that will result in the attenuation of viral pathogenicity.[7] Multiple foreign genes can be inserted into a single viral genome, resulting in an immune response against multiple pathogens.[8] The level of expression of the foreign protein should be high enough to elicit effective immunity. The parental (vector) virus should be tested extensively; its use as a vaccine should be free of side effects. In that regard, the use of the smallpox vaccine strain of vaccinia virus has raised concern with respect to the neurological and dermatological sequelae observed in small numbers of vac-

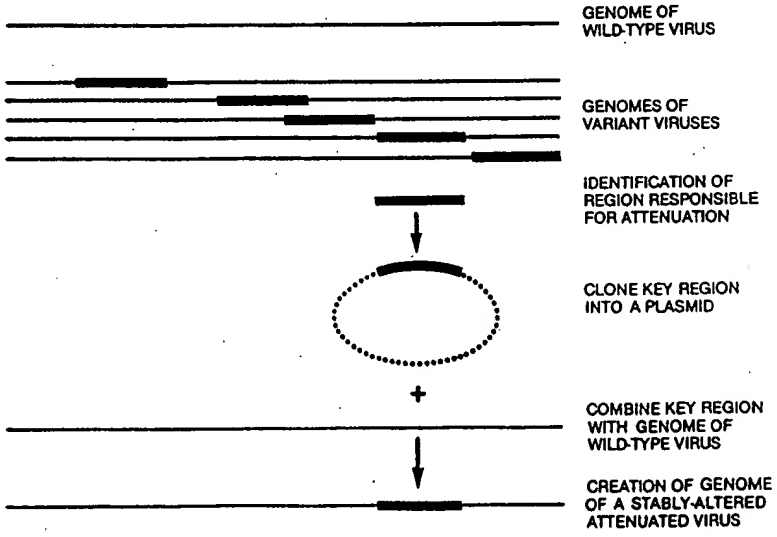


Figure 29–1. Attenuation of viruses using modern techniques in molecular biology.

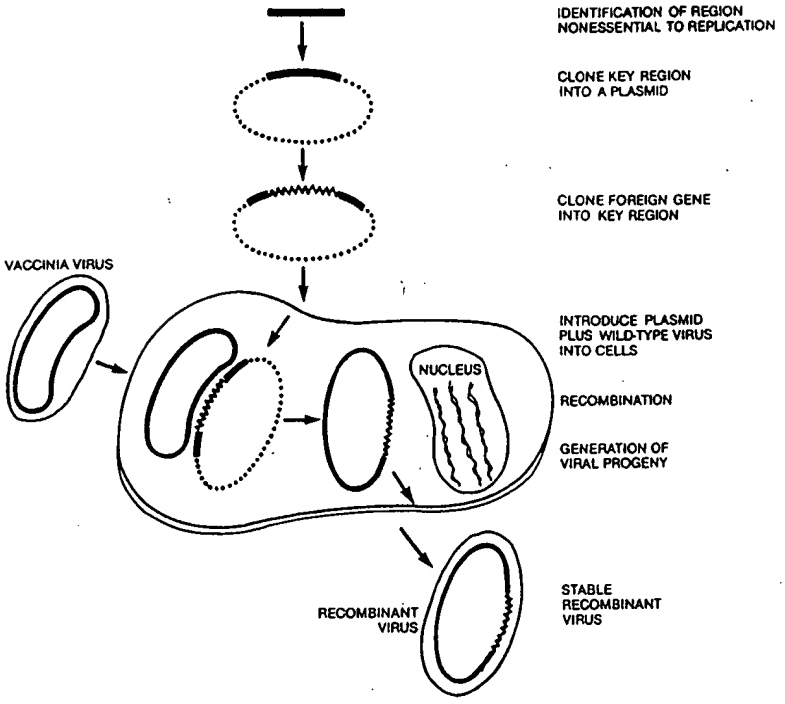


Figure 29–2. Creation of recombinant vaccinia viruses carrying genes that encode immunogens of other pathogens.

cinees. The host range or tissue tropism of the recombinant virus should not be altered significantly compared with that of the vector virus. The effects of viral infection upon the replication and structure of host cells should be studied closely. Since vaccinia virus encodes a protein with significant homology to transforming growth factor-α and to epidermal growth factor (EGF) and since the virus infects cells through the EGF receptor,[9] which is itself highly homologous to the *erb*-B oncogene, there is concern that infection with vaccinia virus may be mitogenic (stimulates growth or division of infected cells). Finally, while some recombinant vaccinia viruses have shown promise in preclinical testing in models of efficacy in animals, only clinical trials and testing of protective efficacy in humans, still awaited, will permit a complete assessment of the utility of such vaccines.

Table 29–3. Considerations in the Safety and Efficacy of Recombinant Live Vaccines

Safety
Extensive testing of parental virus
Stable attenuation of parental virus
Insertion point for the foreign gene
Host range of the recombinant virus
Biology of the cellular receptor for the virus
Efficacy
Multiple foreign genes in a single virus vaccine
Level of expression of foreign protein
Clinical testing

Killed Vaccines

In contrast to live vaccines, killed vaccines do not replicate in the host. Consequently, killed vaccines are often less efficient in the induction of cell-mediated immunity. In order to achieve complete and long-term protection, booster inoculations are required. Furthermore, the greater antigenic mass required for a killed vaccine to be effective, when compared with the antigenic mass for a live vaccine, raises issues of purity. Since they do not replicate, killed vaccines cannot revert to cause clinical disease. Several strategies have been used to develop killed vaccines, as summarized in Table 29–4.

The classic approaches, which employ techniques of biochemical purification and biophysical inactivation, include physical inactivation of whole viruses or bacteria, utilization of inactivated toxoids from bacteria, purification of mon-

Table 29–4. Strategies for the Development of Killed Vaccines

"Classic" Approaches
Killed whole pathogens
Toxoids from pathogens
Purified surface components
Conjugated surface components
"Molecular" Approaches
Recombinant-derived proteins
Synthetic peptides
Anti-idiotypic antibodies

omeric or aggregated surface components of viruses or bacteria and conjugation of surface components of bacteria to other molecules. (These strategies are discussed in greater detail elsewhere.)

The techniques of rDNA have revolutionized biomedical research. They make it possible to identify the gene encoding any protein of interest and to insert that gene into a host cell in such a way that the cell can produce large amounts of the particular protein (Fig. 29–3).

This technology is directly applicable to the development of vaccines. The key to the problem is the identification of that protein component of a virus or microbial pathogen that itself can elicit the production of protective antibodies, such antibodies having the capacity to neutralize infectivity and thus protect the host against attack by the pathogen. The protein then defines biochemical tools for research (e.g., antibodies and amino acid sequences), which are useful for the identification and cloning of the gene encoding that protein. Ultimately, the gene is placed into a host cell in a configuration that will result in synthesis by the host cell of large amounts of the particular immunogenic protein.

The initial application of rDNA technology to the development of vaccines for humans was for the vaccine to prevent infection by hepatitis B virus (HBV). A safe and effective vaccine, consisting of particles of the surface antigen of HBV (HBsAg) has been prepared from human plasma. In order to expand the available supply of vaccine, scientists turned to rDNA technology for vaccine production. The process was initiated by the identification of the gene encoding HBsAg and the insertion of that gene into various host cells. Recombinant yeast synthesize large amounts of particles of HBsAg that are morphologically (Fig. 29–4) and immunologically highly similar to the plasma-derived HBsAg.[10]

Recently, the yeast-derived HB vaccine produced by Merck, Sharp & Dohme became the first rDNA-derived vaccine of any type for humans ever to be licensed anywhere in the world. This prototype vaccine offers hope for the development of a new generation of vaccines, including ones for diseases such as malaria[11, 12] and leprosy[13] for which vaccines cannot be made using classic technologies. The development of recombinant vaccines ultimately may be facilitated by the application of new techniques for the enhancement of the immunogenicity of isolated proteins; one such technique is hydrophobic aggregation.[14] However, because of the biology of the disease or the nature of the immune response induced by the vaccine, it is important to realize that recombinant vaccines do not always provide the solution to the problem of prevention of an infectious disease.

There are a large number of host cells that can be utilized for the production of rDNA-derived proteins. The most common host cells have been bacteria (*Escherichia coli*), yeast (*Saccharomyces cerevisiae*) and mammalian cells (Chinese hamster ovary, monkey kidney). Recently, scientists

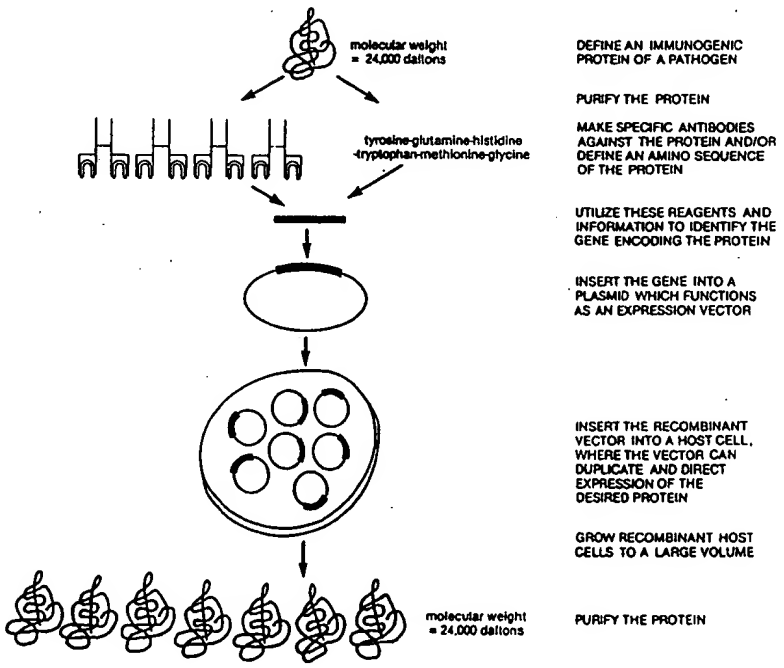


Figure 29–3. The use of recombinant DNA (rDNA) technology to express large amounts of a desired protein.

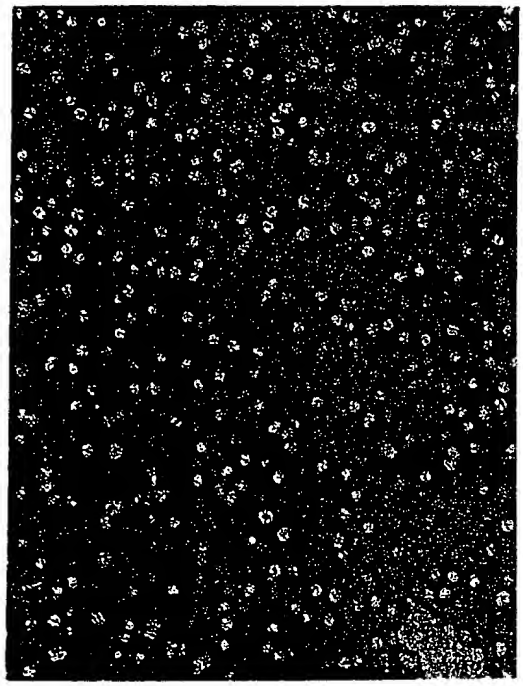


Figure 29–4. Electron micrograph of particles of HBsAg produced by recombinant yeast (165,000X). (Courtesy of B. Wolanski, Merck Sharp & Dohme Research Laboratories.)

have diversified to the use of other bacterial (*Bacillus subtilis*), fungal (*Aspergillus nidulans*) and higher eukaryotic (insert) cells. All these systems can be judged by a wide range of criteria relating to desirable traits of either the protein product or the host cell as well as to safety considerations (Table 29–5).

The most commonly employed expression systems can be evaluated relative to one another

Table 29–5. Expression Systems for rDNA-derived Proteins

Desirable Traits of the Product
High yields (commercial)
Stability of yield with scale-up of cells
Inducible expression
Secretion
Post-translational modifications (consistent with immunogenicity)
Glycosylation
Phosphorylation
Amidation
Carboxylation
Hydroxylation
Proteolytic processing
Desirable Traits of the Host Cells
Ease of scale-up
Consistency of performance
Lack of oncogenic elements
Rapid division
Safety Concerns
Heterologous protein contaminants
Biology of cell substrate
Residual DNA (oncogenesis)

*Scheme to take cultures from bench to large-scale fermentation or purification.

with respect to each of these criteria (Table 29–6). These criteria fall into three groups, which roughly discriminate between the microbial (yeast and bacteria) and mammalian expression systems as follows:

1. The microbial systems are more productive and consistent in overall performance than the mammalian ones.

2. Mammalian cells provide for post-translational modifications that often resemble more closely those in the viral agent than those provided by microbial cells.

3. With few exceptions, serially propagated

Table 29–6. Comparison of Commonly Used Expression Systems for rDNA

	E. coli (Bacteria)	*S. cerevisiae* (Yeast)	Chinese Hamster Ovary (Mammalian Cells)
Yield of product	+ + +	+ + +	+
Ease of scale-up	+ + +	+ + +	+
Stability of yield with scale-up	+ + +	+ + +	+
Inducible expression	+ + +	+ + +	+
Consistency of performance	+ + +	+ + +	+
Secretion	+	+ +	+ + +
Glycosylation	–	+ +	+ + +
Proteolytic processing	–	+ +	+ + +
Other modifications	–	+ +	+ + +
Biology of cell substrate	+ +	+ + +	+
Heterologous protein contaminants	+ +	+ +	+
Residual DNA	+ + +	+ + +	+

+ + + = most acceptable
+ + = acceptable
+ = least acceptable
– = absent

mammalian cells, unlike microbial cells, are considered "transformed," meaning that they are more susceptible to oncogenicity in experimental animals.

Furthermore, for expression of rDNA, mammalian cells often utilize genetic elements derived from oncogenic or latent viruses, while microbial cells do not utilize such elements. These perceived safety concerns must be addressed regarding the use of mammalian cells as an expression system for recombinant vaccines.

These relative evaluations represent generalizations from a large number of studies in the different systems and should be considered whenever an expression system is utilized. Nevertheless, each attempt at expression must be evaluated individually, and there are probably as many exceptions as there are rules in the "expression game"!

The use of synthetic peptides as vaccines involves the use of short segments of a protein molecule, rather than the entire molecule, as the immunogen. Some peptides are able to induce antibodies that can react with the whole protein as well as with the peptide per se.[15] The discovery process for formulating synthetic peptide antigens begins by defining the gene encoding the immunogenic protein (see Fig. 29–3), then branches off by exploiting the DNA sequence of the gene to define the amino acid sequence of the protein and to predict which regions of the protein might be immunogenic (Fig. 29–5). Once defined, peptides can be synthesized chemically[16] and formulated into synthetic vaccines.

This approach first was applied to the development of vaccines for humans by synthesizing portions of the HBsAg polypeptide.[17] In theory, the approach is technically versatile and lends itself to the production of well-defined vaccines. However, in practice, the approach has several shortcomings relative to the use of whole proteins. In general, the antibodies elicited by an intact protein crossreact more effectively with both the protein and the pathogen on which it resides than do antibodies elicited by a synthetic peptide. Furthermore, such antibodies bind with higher affinity and are present at a higher titer than are those elicited by the peptide. Thus, the duration of the immune response stimulated by a synthetic peptide is inferior to that stimulated by a whole protein. At minimum, a complete cocktail of synthetic peptides may be required as well as an improvement in methods for the enhancement of immunogenicity by covalent conjugation onto carrier proteins. It may be that synthetic peptides, however tailored, cannot mimic all the conformations assumed by the

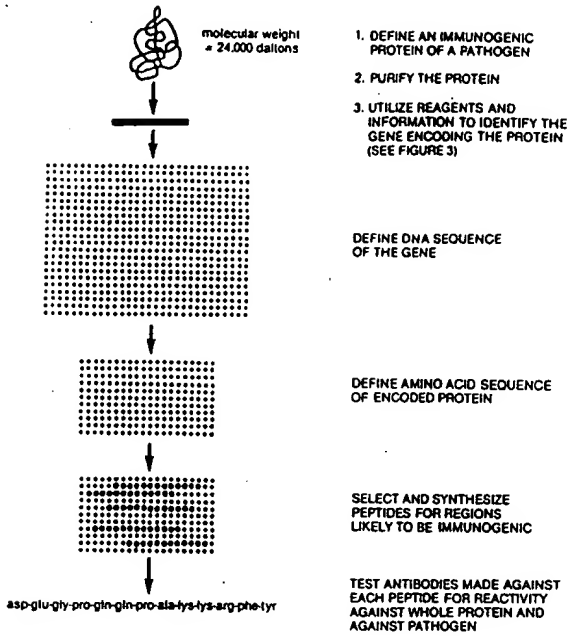
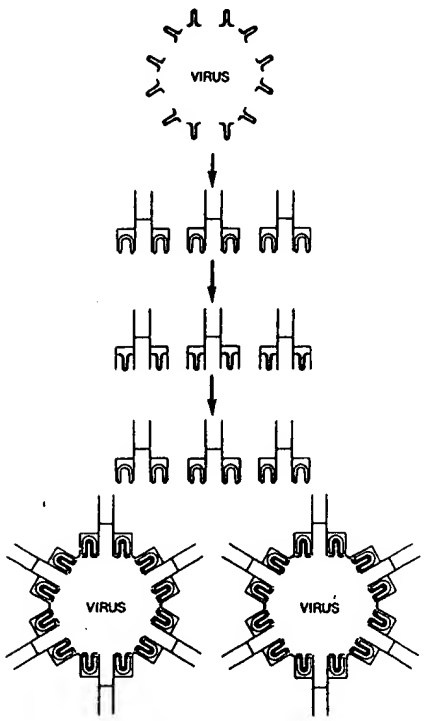


Figure 29–5. Defining immunogenic peptides from immunogenic proteins.

intact protein that are critical for immunogenicity. Furthermore, immunogens often have complex chemical structures, e.g., sugars and lipids, which cannot be specifically applied to a synthetic peptide. However, synthetic peptides may be useful for the priming of an immune response, as first demonstrated for poliovirus.[18]

A third novel strategy for the formulation of killed vaccines is the use of anti-idiotypic antibodies (anti-antibodies), whose existence and function in the regulation of the immune response first were articulated by Jerne.[19] Since antibodies bear a structural image of the primary antigen at the antigen-combining site (idiotype), antibodies to antibodies (anti-idiotype) have an antigen-combining site that is structurally similar to the antigen (Fig. 29–6). Thus, inoculation of the anti-idiotypic antibody functions as a vaccine by inducing an anti-anti-idiotypic antibody which in principle should be identical to the first antibody.[20]

This approach has been applied to formulating a vaccine for hepatitis B.[21] While this strategy clearly warrants further study, it suffers from two potential drawbacks. Since the immunogen is an antibody, which is structurally related to naturally occurring human antibodies, problems related to antigenic sensitization must be addressed. In addition, the images borne by anti-idiotypic antibodies are structurally analogous to peptide domains on the surface of the pathogen rather than to whole proteins. Therefore, such



IMMUNOGENIC STRUCTURE (ℐ) ON A VIRUS

VIRAL INFECTION OF A HUMAN

ANTI-VIRAL ANTIBODIES

INOCULATE ANTI-VIRAL ANTIBODIES INTO ANIMALS

ANTI-IDIOTYPIC ANTIBODIES

PURIFY AND INOCULATE ANTI-IDIOTYPIC ANTIBODIES INTO HUMANS AS A VACCINE

ANTI-ANTI-IDIOTYPIC ANTIBODIES ARE ANTI-VIRAL ANTIBODIES

Figure 29–6. Strategy for the use of anti-idiotype antibodies as yaccines.

vaccines might elicit an immune response which is more antipeptide-like in nature rather than antiprotein-like, as discussed previously.

The progression of a vaccine candidate from the laboratory to the marketplace is long and arduous, often taking 10 years from the time of its initial discovery and characterization. Vaccines made by means of new molecular technologies are being developed rapidly. Of these, only rDNA-derived proteins have gone as far as human clinical trials, much less having become a licensed product as in the case of yeast-derived HBsAg. Representatives of such vaccines are listed in Table 29–7, along with others derived by older types of technologies.

With the development of the increasingly so-

Table 29–7. The Progression of Human Vaccines Made by Different Technologies Toward Becoming Licensed Products

	Preclinical Testing	Clinical Testing	Licensed Product	Examples
Live Vaccines				
Classic Strategies				
Modification in cell culture	X	X	X	Measles, mumps, rubella
Variants from other species	X	X	X	Smallpox (vaccinia), rotavirus
Temperature-selected mutants	X	X		Influenza
Reassorted genomes	X	X		Rotavirus
Molecular Strategies				
DNA modification mutants	X			Poliovirus, *Salmonella, Shigella*
Recombinant viruses	X			Vaccinia, herpes simplex, varicella-zoster
Killed Vaccines				
Classic Strategies				
Killed whole pathogens	X	X	X	Pertussis
Toxoids from pathogens	X	X	X	Diphtheria, tetanus, cholera
Purified surface components	X	X	X	Hepatitis B
Conjugated surface components	X	X		Meningitis (*Hemophilus influenzae* b)
Molecular Strategies				
Recombinant-derived proteins	X	X	X	Hepatitis B
Synthetic peptides	X			Hepatitis B
Anti-idiotypic antibodies	X			Hepatitis B, rabies

phisticated analytical tools of molecular biology and immunology, vaccines derived from the newer technologies are receiving closer scrutiny at the regulatory and clinical levels than have vaccines derived from more classic strategies. This trend is expected to continue and represents a formidable barrier for manufacturers to hurdle with respect to the licensing of safe and effective vaccines. As with any technology, there is a learning curve for both manufacturers and regulatory agencies.

A major challenge facing manufacturers and society in the United States is the profound increase in litigation over adverse experiences related to vaccines. The most dramatic manifestation of this litigation is the increased expense and intermittent unavailability of product liability insurance to the three United States–based manufacturers of the vaccine for pertussis, thus resulting in the temporary withdrawal of products of two of these firms from the market and the tripling in the price of the vaccine. Medically, one may find a situation in which the general welfare of the pediatric population may be at significant risk to whooping cough. This problem could have tragic consequences for society and become a severe disincentive for the development of vaccines by means of new technologies. It is hoped that, while legislative remedies to this severe problem are being addressed, research scientists and medical researchers will continue to receive as much support as possible in the pursuit of new technologies, since the vaccines that result represent the most cost-effective products for the eradication of infectious diseases.

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PLOTKIN & MORTIMER

VACCINES

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DEVELOPMENT OF AN ANIMAL MODEL TO TEST A HELICOBACTER-PYLORI VACCINE
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DEVELOPMENT OF AN ANIMAL MODEL TO TEST A *HELICOBACTER PYLORI* VACCINE

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An animal model is being developed to test a vaccine against *H. Pylori* and to investigate the mechanism of the immune response to *H. Pylori* in the gut.

Initially, rats were immunized using several different immunization regimes, viz. intramuscular (IM) immunization with lyophilized *H. Pylori* in Freunds' complete adjuvant (FCA), intra-Peyers patch (IPP) immunization with paraformaldehyde-killed *H. Pylori* in FCA and oral immunization with live *H. Pylori*, lyophilized *H. Pylori* or paraformaldehyde-treated *H. Pylori*, all in phosphate buffered saline and with prior administration of sodium bicarbonate.

IM immunization produced a significant enhanced IgG and IgA *H. Pylori* specific antibody response in the serum but had no effect on the salivary antibody response. IPP immunization gave an enhanced serum and saliva IgG and IgA antibody response and the Peyers patch lymphocytes were demonstrated to have a substantial proliferative response to a crude *H. Pylori* antigen preparation in vitro (stimulation index=64 $\pm$ 26) indicating that the gut mucosa is capable of mounting a vigorous immune response against the *H. Pylori* bacteria. Oral immunization however, has been less successful. Oral immunization with live or para-formaldehyde-treated bacteria gave no significant enhancement of the serum or saliva anti-*H. Pylori* antibody. Oral immunization with lyophilized H. *Pylori* gave a small increase in serum antibody response but this was not significant. The enhancement of this response by the addition of adjuvants is under investigation.

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H2-4

IMMUNISATION AND GASTRIC COLONISATION WITH *HELICOBACTER FELIS*

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Introduction

There is accumulating evidence that long term infection with *Helicobacter pylori* is a prerequisite for the development of atrophic gastritis and the subsequent development of gastric cancer in a subset of persons in certain developing countries. Thus, introduction of intervention strategies at an early age may influence the morbidity and mortality of this serious disease. Immunisation would be an attractive option but, given *H. pylori* can survive in the body for tens of years in the presence of a strong immune response, may not be effective. *Helicobacter felis* will colonise the gastric mucosa of SPF mice in large numbers occupying the gastric pits and mucus. Like *H. pylori* in humans this bacterium will remain for the life of the animal. Thus, the *H. felis*- infected mouse would appear to be a good model to test the hypothesis that immunisation can protect against colonisation with gastric helicobacters.

Methods

SPF mice were immunised by intravenous injection of 0.1 ml of a suspension of viable *H. felis* (10^8 / ml) once a week for 5 weeks or infected *per os* over 5 days with three doses of the bacterium. Immune responses of both these groups of animals were measured. A similar group of parenterally immunised animals were challenged with living cultures of *H. felis*. A final group of orally *H. felis* -infected animals was cleared of the organism with triple anti-microbial therapy for 28 days (tetracycline, metronidazole, bismuth subcitrate). These animals and controls that had been given saline instead of triple therapy were then challenged with a living culture of *H. felis*. All challenged animals were assessed for *H. felis* colonisation by rapid urease testing of gastric tissue and histology.

Results

Parenteral immunisation of mice with living cultures of *H. felis* induced a very high level of serum IgG, significant IgM and IgA could be detected in the bile. Serum responses post oral infection were much less and developed slowly. Hyperimmunisation of mice with an intravenous injection of a live culture of *H. felis* had no protective effect on gastric colonisation. In contrast, in mice cleared of infection with *H. felis* by administration of a one month treatment of antibiotics, some effect on rechallenge was seen. Colonisation was significantly delayed, with numbers of animals showing no urease reactivity for up to 10 days after rechallenge with an inoculum of *H. felis* that always gave 100% positivity in normal animals.

Conclusion

Parenteral immunisation with *H. felis* gave absolutely no protection against gastric colonisation. The same is likely to be true for *H. pylori*. However, preliminary experiments show that previous oral infection with living bacteria did appear to have some effect on reinfection. Further experiments are in progress to assess the value of oral immunisation against infection with gastric helicobacters.